Low protein diet induced anti-cancer immune response

Józef Piotr Bossowski

UNIVERSITÉ CÔTE D'AZUR

ÉCOLE DOCTORALE
SCIENCES DE LA VIE ET DE LA SANTÉ

THÈSE DE DOCTORAT

Induction d'une réponse immunitaire anti-tumorale par un régime pauvre en protéines

Low protein diet induced anti-cancer immune response

Józef Piotr BOSSOWSKI

Equipe 3 : Metabolism, cancer and immune response

Présentée en vue de l'obtention du grade de docteur en Sciences de la Vie et de la Santé, Spécialite :Interactions moléculaires et cellulaires d'Université Côte d'Azur
Dirigée par : Dr. Jean-Ehrland RICCI
Soutenue le : 6 décembre 2018 à Nice, C3M Bâtiment Archimède, Hôpital de l'Archet 2

Devant le jury, composé de :
Dr. Béatrice BAILLY-MAITRE, Présidente du Jury, C3M
Dr. Serge MANIE, Rapporteur, CRCL
Pr. Philippe NAQUET, Rapporteur, CIML
Dr. Eric CHEVET, Examinateur, OSS CLCC
Dr. Jean-Ehrland RICCI, Directeur de Thèse, C3M

SIGNALIFE

Inserm U1065

UNIVERSITÉ CÔTE D'AZUR

ÉCOLE DOCTORALE
SCIENCES DE LA VIE ET DE LA SANTÉ

THÈSE DE DOCTORAT

Induction d'une réponse immunitaire anti-tumorale par un régime pauvre en protéines

Low protein diet induced anti-cancer immune response

Józef Piotr BOSSOWSKI

Equipe 3 : Metabolism, cancer and immune response

Présentée en vue de l'obtention du grade de docteur en Sciences de la Vie et de la Santé, Spécialite :Interactions moléculaires et cellulaires d'Université Côte d'Azur
Dirigée par : Dr. Jean-Ehrland RICCI
Soutenue le : 6 décembre 2018 à Nice, C3M Bâtiment Archimède, Hôpital de l'Archet 2

Devant le jury, composé de :
Dr. Béatrice BAILLY-MAITRE, Présidente du Jury, C3M
Dr. Serge MANIE, Rapporteur, CRCL
Pr. Philippe NAQUET, Rapporteur, CIML
Dr. Eric CHEVET, Examinateur, OSS CLCC
Dr. Jean-Ehrland RICCI, Directeur de Thèse, C3M

SIGNALIFE

Inserm U1065

Résumé

Plusieurs arguments de la littérature suggèrent l'importance de l'alimentation dans le développement tumoral et l'efficacité des traitements anti-cancereux. Dans différents modèles animaux, la restriction calorique (CR) supprime la prolifération des cellules tumorales et les sensibilise aux thérapies ciblées. Par conséquent, des approches non-pharmacologiques comme la restriction calorique ont un intérêt grandissant en clinique.

Considérant l'addiction des cellules tumorales aux nutriments, nous nous sommes demandé quels macronutriments pouvaient avoir des propriétés anticancéreuses. A partir d'un modèle murin de lymphomes B (modèle transgénique Eμ-Myc) nous avons testé l'impact de deux régimes alimentaires : l'un pauvre en glucides (Low CHO, 25% de réduction en glucides) et l'autre pauvre en protéines (Low PROT, 25% de réduction en protéines). Des souris syngéniques C57BL/6 ont été injectées par voie intraveineuse avec des cellules primaires Eμ-Myc. Malgré un apport alimentaire équivalent entre les groupes, nous avons observé que le régime pauvre en protéines augmente la survie globale des souris C57BL/6 développant un lymphome B Eμ-Myc. De manière intéressante, nous avons démontré que cet effet pro-survie est dépendant du système immunitaire. En effet, la déplétion des cellules T $CD8^+$ ou l'utilisation d'un modèle murin immunodéficient NSG (NOD-SCID il2rγ), empêche l'effet bénéfique du régime pauvre en protéines sur le développement tumoral. Nous avons reproduit et étendu nos observations en utilisant des lignées modèles de cancéreuses colorectaux (CT26) et de mélanome (B16) injectée dans des souris syngéniques, immunocompétente.

Les cellules tumorales étant fortement dépendantes des nutriments, nous avons émis l'hypothèse qu'un régime pauvre en protéines pourrait induire un stress du réticulum endoplasmique (RE) dans ces dernières. En effet, nous avons observé une augmentation des protéines impliquées dans la signalisation du RE : CHOP et sXBP1. Par conséquent, nous avons traité les souris nourries en régime pauvre en protéines avec deux inhibiteurs du stress du RE : TUDCA, inhibiteur générique et MKC4485 qui cible l'activité ribonucléase d'IRE1. Dans les deux cas, ces inhibiteurs ont bloqué l'effet du régime faible en protéines sur le développement tumoral et l'infiltration des T $CD8^+$ au sein de la tumeur. Pour s'affranchir, des potentiels effets secondaires des inhibiteurs chimiques, nous avons invalidé IRE1 dans la lignée CT26 et nous avons obtenus des résultats similaires, démontrant que la voie IRE1 dans les cellules tumorales est une voie centrale dans la réponse immunitaire anticancéreuse induite par un régime pauvre en protéines. En outre, nous avons découvert que l'activation de RIG-I est un événement en aval de l'activation d'IRE1 et que, par analyse bio-informatique nous avons pu corréler une signature IRE1 à une infiltration immunitaire élevée et à une immunogénicité accrue du cancer chez les patients atteints de mélanome, glioblastome et cancer colorectal. De ce fait, nous avons démontré que la réponse du système immunitaire induite par un régime pauvre en protéines est une conséquence de l'activation accrue de IRE1 dans les cellules cancéreuses.

Mots clés : IRE1, RIG-I, réponse immunitaire, stress du réticulum endoplasmique, cancer.

Dla moich rodziców

"In the middle of difficulty lies opportunity"

Albert Einstein

Acknowledgements

I would like to thank all the members of the jury for evaluating my work. I am grateful to **Professor Philippe Naquet** and **Doctor Serge Manié** for reading and correcting my manuscript. Doctor **Eric Chevet** for accepted to be my examiner and for advising and helping during the development of my work. Warm thanks to **Doctor Béatrice Bailly-Maitre** for accepting to be the jury president and having always time for scientific discussion when we needed it.

Very special thanks to **Doctor Jean-Ehrland Ricci**, my boss and PhD director. Thank you for giving me this outstanding opportunity to join your lab and supervising me over these 4 years of my scientific development. Thank you for keeping your promise and providing me an laboratory environment that helped me grew scientifically promoting all range of skills, from lab techniques to oral presentations and critical thinking. Thank you for the many occasions you spent your time discussing and explaining scientific issues that true value I still keep discovering along my work. Thank you for being there for many times we celebrated success and the times we needed your help and support encountering difficulties.

Gracias **Camila** !!! I should write separate chapter if I would like to include all of the things I wish to thank you for. Except for the obvious one, as introducing me to almost all the laboratory techniques, mentoring me and working on the common project. On top of all that, I'm thankful to you especially for being my dear friend that I could rely on in every time. There is absolutely no way I could have managed all the work we have done without your enthusiasm, support and the amazing atmosphere we had. I will always remember your determination and passion you had pursuing scientific goals and problems. Thank you for your friendship, all the times we spend partying, drinking and talking. For surviving together through good and bad times! I'm so happy for you pursuing your dreams, together with you husband and cats. And speaking of him, all the best **Nico**! Thank you for letting me visit you so many times, very often overstaying your welcome at your house ;) Thank you for all the amazing times we had together, I wish you many successes and cheerful moments on "another continent". I would say that I will miss you both enormously, but it seems like we are going to see each other more often than anyone would expect in the big apple!

Merci **Elo**. Merci for your friendship, help and support during all of these years. It was a pleasure working next to you. I wish you all the best for the future, you definitely deserve it! Stay the person you are, because you are awesome! See you soon and hopefully many many times in the following years. There are a lot of Moscow mule waiting for us.

Merci **Ranana**. You are next one. Stay strong, your hard work will pay off and you are example of an excellent PhD student. I cannot know how many sacrifices you had to do to and the determination you have to month after month give 120% of you. You are close to accomplish your goals, and you can be very proud of that!

Adriana, the best I can do is to wish you all the strength and luck and scientific support that you can get because I know how big and difficult challenge is in front of you. You already

proved yourself as a hard work person who have sharp mind and critical scientific thinking, indispensable for good PhD student. Thank you for all the help and engagement you put into proofreading my manuscript. I wish you many successes in your professional as well as private life!!! And I hope we will keep in touch.

Romain, my dupeczka… It was a pleasure having you in the lab, even if you just dropped for a second to have a nap ;) but seriously, I admire your determination and hard work, and on the other hand the party spirit and joy of life you have. Hope we will meet again, either in France, Poland, US, or anywhere else the life will decide to put us. Best of luck man.

Benjamin, thaaaaaannnnkkkk youuuuu b…. . You've told us that just yesterday Léo was born. Congratulations man, to you and Christelle! You will be amazing father, and if you managed to pass even half of your craziness unto your son I'm sure he will be a great guy, unfortunately likely to make his parents crazy from time to time as well :D

Merci to all the team 3 members, the current one and the former: **Emma** (I could always count on you and your resourcefulness), **Johanna** (for all the discussion we had and many many advices), **Els** (your scientific expertise and knowledge helped us soooo many times, and you were always eager to help whenever we needed! thank you), **Florian** (I wish you all the best man, you remember, don't stop taking pictures, they are awesome), **Laura** (I miss having you in the lab, it was never the same place as it was when you were around), **Sandrine**, **Manuel**, **Ashaina**, **Blandine**, **Rachel**, **Barbara**, **Amani**. During my PhD I worked directly with people from at least 8 countries. What are the chances we would have met in one lab during these years and that would result in such unique and amazing time?! Thank you all for that.

Ciccio, GMDD, I was so sad when you have left. I miss our discussions and your optimism. It was a pleasure working with you and learning from you! Thank you ciccio.

Lázaro, my Cuban bro! You were literally the first person I have met when I arrived to Nice, never would have imagined we are gonna end up working in neighbouring offices. I wish you and Yaidelys many happy years ahead.

C3M facility managers and offices: **Virginie** and **Dominique**, for all the problem they had with my poor knowledge of French, and yet they managed to help me with so many situations. **Philippe**, always busy but helpful and fast when necessary. **Maeva** – microscopy platform, amazing job and wonderful personality, I wish you all the best and thank you for all your help. Special thanks for **Vero** and whole animal facility **team**!!! Vero, for your professionalism and help in critical situations – thank you, thank you, thank you! And **Nicolas**, I wish to have such amazing bioinformatician in my future work to help me sort out through the data.

All the people in C3M that I encounter during my years of PhD. There were so many of you that helped me at so many steps!!! Thank you for all the help and that I could always count on it when I needed.

Thanks to all Labex-SIGNALIFE members, and especially to **Konstanze**, your help was indispensable for my whole PhD. Its solely because of this program I had chance to meet so

many incredible people from all around the worlds I shared many wonderful moments. I wish all the members the best of luck and successful continuation of your scientific career.

My friends I have met in Montpellier. **Solange**, because of you I appreciated France so much I ended up in Nice. You are amazing person, caring so much for all the people you work with and so passionate about science that it inspires all people around you. It was an honour and a great pleasure working with and from you. And many many thanks to the people I met and work with from your lab; **Basia**, **Brusia**, **Irena** and **Stephan**. Thank you for everything!

Serdeczne podziękowania księdzu **Bronisławowi**. Bez jego wsparcia i modlitwy ta praca i mój pobyt w Nicei nie byłby tak owocny. Szczęść Boże za całą księdza pracę i wysiłek w utrzymaniu prawdziwie polskiej parafii na obczyźnie. Za cały księdza entuzjazm i pozytywne nastawienie do życia oraz gotowość do pomocy ludziom kiedy tylko ksiądz zdoła, a kiedy tak często jest to niedostrzegane. Z całego serca Bóg zapłać i Szczęść Boże.

Moim przyjaciołom których zostawiłem w Polsce. **Sylwi** i **Maćkowi**. **Piotrkowi** i **Ani**. **Piotrkowi**, **Kamilowi**, **Marcinowi**, serdeczne pozdrowienia i podziekowania za pamięć o mnie, i nieustanne próby sprowadzenia mnie z powrotem! Część mnie zawsze będzie w Polsce, I ta część zawsze będzie myślami z każdym z was. Dziękuję wam za całe wsparcie I chwile które razem spędziliśmy dyskutując o wszystkim co jest w życiu ważne. Dziękuję że zawsze mogę na was liczyć. W anglii – **Gabi** and **JP** – best kisses, I'm so happy for you and missing you enormously.

Serdeczne podziękowania dla mojej byłej szefowej, wspaniałej profesor **Joannie Cichy** i całego jej laboratorium ktore wprowadziło mnie w świat nauki: **Asi**, **Mateuszowi**, **Agnieszce**, kolejnej **Asi**, **Kasi**, i wszystkim innym których poznałem w laboratorium Immunologii Uniwersytetu Jagiellońskiego. Gdyby nie wasza pasja i wspaniałe podejście do życia (ktorego zdecydowania zbyt często brakuje mi poza krajem) nie byłoby mi łatwo znaleźć to co tak lubię robić w życiu.

Merci **Samia**. I have spent far to less time with you than I would like to, because you are wonderful person. Thank you for all the kindness and understanding you are giving me. Thank you for all the occasions we met in Nice and in Paris. Thank you for your commitment to show and share your culture with me. Thank you for your sympathy and for all the discussion we had. I very appreciate that.

Very special thanks to my dearest **Marwa**, my cukiereczek. I cannot stress enough how special person you are and how big place you have in my life! I'm so happy I met you and you allowed me to be a part of your life. Thank you for making me a better person, despite my stubbornness and tete dur. Thank you for all the times you forgave me for upsetting you when I was to blind to see my own mistakes. Thank you for all the discussion we had, and your support whenever I needed it. You are so amazing, cheerful, sincere, understanding, thoughtful, I enjoy every moment I spent with you. Thank you for that!!!

To my **Family**. Moim młodszym siostrom **Joannie** i **Annie**, Asi i Ani, za wszystkie chwile wspólnie spędzone, za wszystkie odwiedziny i kontakt i pamięć. Życzę wam samych sukcesów i dziękuję za to że mogę na was zawsze liczyć.

Moim najbliższym, kochanym Rodzicom, Jadwidze i Piotrowi, **Mamie** i **Tacie**. Dziękuję wam za nieustanne wsparcie i wiarę we mnie, na każdym etapie mojego życia. Nigdy nie zdołam się wam odwdzięczyć za wszystko co dla mnie zrobiliście i dalej robicie. Dziękuję za zrozumienie i wsparcie mojego wyjazdu, chociaż odległość bywa męcząca. Dziękuję za to jacy jesteście, to wasz wysiłek i charakter jest przykładem dla mnie. Dziękuję wam za to z całego serca.

Table of Contents

Abbreviations

ABC	Activated B cell-like
2DG	2-deoxyglucose
acetyl-CoA	acetyl coenzyme A
AICAR	5-Aminoimidazole-4-carboxamide ribonucleotide
AMPK	AMP-activated protein Kinase
APCs	Antigen-Presenting Cells
ATF6	Transcription Factor 6
ATF6	Activating transcription factor 6
BAK	Bcl-2 homologous antagonist killer
BAX	Bcl-2-associated X protein
BCL_XL	B-cell lymphoma-extra large
BID	BH3 interacting domain death agonist
BIM	Bcl-2-like protein 11
CR	Caloric Restriction
CRC	colorectal carcinoma
CRm	Caloric Restriction mimetics
CRT	Calreticulin
CSF1	Colony-stimulating factor 1
CT26	Mus musculus colon carcinoma
CTLA4	Cytotoxic T-Lymphocyte Associated Protein 4
CXCL-	The chemokine (C-X-C motif) ligand -
DAMPs	Danger-Associated Molecular Patterns
DCs	Dendritic cells
DD	Death Domain
DLBCL	Diffuse Large B cell Lymphoma
DSR	Differential-Stress-Resistance
DSS	Differential-Stress-Sensitization
eEF2K	eukaryotic Elongation Factor 2 Kinase
ER	Endoplasmic Reticulum
ERAD	ER-Associated Degradation
FADD	Fas-associated protein with death domain
FAS	Fatty Acid Synthase
FASL	FAS Ligand
FMD	Fasting Mimicking Diet
GCB	Germinal Center B cell-like
GF	Geometric Framework
GH	Growth Hormone
GLS1	Glutaminase 1
HCC	Hepatocellular Carcinoma
HDACi	Histone Deacetylase Inhibitors
HIF-1α,	Hypoxia Inducible Factor-1α
HMGB1	High mobility group box 1 protein

HMGB1	High Mobility Group Box 1
HSP	Heat Shock Protein
ICD	Immunogenic Cell Death
IDO1	Indoleamine 2,3-Dioxygenase 1
IFN-I	type I-Interferons
IGF-1	Insulin-like Growth Factor 1
IGFBP1	Insulin-like Growth Factor Binding Protein 1
IRE1	Inositol Requiring Enzyme 1
Low CHO	Low Carbohydrate diet
Low PROT	Low Protein diet
LPS	Lipopolysaccharide
MCL-1	Myeloid Cell Leukemia-1 protein
MCP-1	Monocyte Chemoattractant Protein-1
MDSCs	Myeloid-Derived Supressor Cells
MHC-I and II	Major Histocompatibility Complex class I and II
MOM	Mitochondrial Outer Membrane
MOMP	Mitochondrial Outer Membrane Permeabilization
mTOR	mammalian Target of Rapamycin
mTORC1	mammalian Target of Rapamycin complex 1
MTX	mitoxantrone
NAD	nicotinamide adenine dinucleotide
NK cells	Natural Killer cells
NO	Nitric oxide
NSAID	nonsteroidal anti-inflammatory drugs
OxPhos	Oxidative Phosphorylation
PAMPs	Pathogen-Associated Molecular Patterns
PD-1	Programmed cell death protein 1
pDC	Plasmacytoid dendritic cell
PD-L1	Programmed cell death protein 1 Ligand
PERK	PKR-like Endoplasmic Reticulum Kinase
PHD1	Prolyl Hydroxylase 1
PI3K	Phosphoinositide 3-kinase
PKB	Protein kinase B (or AKT)
PRRs	Pattern Recognition Receptors
PTEN	Phosphatase and tensin homolog
RB	Retinoblastoma protein
RENCA	kidney renal adenocarcinoma
RIDD	Regulated IRE1-Depedent RNA Decay
RIG-I	Retinoic acid-Inducible Gene-I
RLRs	Retinoic-acid-inducible gene-I-Like Receptors
ROS	Reactive oxygen species
S1P/S2P	Site-1 and Site-2 Proteases
SCD1	Stearoyl-CoA Desaturase
SOD2	Superoxide dismutase 2
TAMs	Tumor-Associated Macrophages
TCA	The tricarboxylic acid cycle

TCR	T Cell Receptor
TERS	transmissible ER stress
TERT	Telomerase reverse transcriptase
TILs	Tumor Infiltrating Lymphocytes
TLR	Toll-like Receptors
TME	Tumor Microenvironment
TNBC	triple negative breast cancer
TRAIL	TNF-related apoptosis-inducing ligand
Tregs	regulatory T cells
TSC	Tuberous sclerosis protein
TUDCA	Tauroursodeoxycholic Acid
UPR	Unfolded Protein Response
UPR	Unfolded protein response
UV	Ultraviolet

List of Figures

List of Tables

SUMMARY

Several arguments from the literature suggested the importance of diets in cancer development and in the efficacy of anti-cancer therapies. Calorie restriction (CR) suppresses cancer growth in various animal models and sensitizes tumor cells to targeted therapies (Meynet & Ricci, 2014). Thus, non-pharmacologic approaches such as CR have a growing interest in the clinic.

Considering the nutrient addiction of cancer cells, we wondered which specific macronutrients contribute the most to anti-cancer effects. Therefore, we tested the reduction in specific macronutrient without decrease in general calorie intake on tumor development. We used two diets: reduced in carbohydrates (Low CHO, -25% carbohydrates) and diet reduced in protein (Low PROT, -25% proteins) on the Eμ-Myc transgenic mouse model of B-cell lymphoma. Syngeneic C57BL/6 mice were intravenously injected with primary Eμ-Myc cells. We observed that low PROT-diet, in spite of equal calorie intake among the groups, resulted in increase of the overall survival of Eμ-Myc-bearing C57BL/6 mice. Very importantly, we established that this pro-survival effect is immune system-dependent as both depletion of $CD8^+$ T cells and use of immunodeficient NSG (NOD-SCID il2rγ) mouse model prevented the beneficial effect of the low PROT-diet on the tumor development. We reproduced and further extended our observations using subcutaneous injection of CT26 colorectal cancer cells in syngeneic immunocompetent BALB/c mice and B16 melanoma in C57BL/6 mice.

As tumor cells are highly dependent on nutrients, we speculated that low PROT diet could induce ER stress in tumor cells. Indeed, we observed increase in proteins implicated in ER stress signaling – CHOP and sXBP1. Therefore, we treated low PROT-diet fed mice with two ER stress inhibitors, the general inhibitor TUDCA or MKC4485, which targets IRE1 RNAse activity. In both cases, inhibitors significantly prevented the effect of the Low PROT-diet on tumor development and on intratumoral number of $CD8^+$ T cells. To eliminate any side effects of chemical inhibitors, we invalidated IRE1 in CT26 cells and obtained similar results, demonstrating that IRE1 signaling in tumor cells is a central event in the low PROT-diet induced anti-cancer immune response. In addition, we have uncovered RIG-I activation as a downstream event of IRE1 activation and by bioinformatic analysis correlated high-IRE1 signature with high immune infiltration and enhanced immunogenicity of cancer in patients bearing melanoma, glioblastoma and colorectal cancer. Hence, we have shown that the immune system response elicited under a Low PROT diet is a consequence of increased IRE1 activation in cancer cells.

INTRODUCTION

Cancer has probably accompanied multicellular organisms since the very early beginning of their evolutionary development. Archeo-biologists have found fossils of dinosaurs already affected by tumor (Dumbrava et al., 2016; Rothschild, Witzke, & Hershkovitz, 1999). Similarly, our direct Neanderthal ancestors 150 000 years ago already had incidents of cancer (Monge et al., 2013). Multiple malignant tumors have been found in Egyptian mummies, and even more interestingly, first written description of breast cancer comes from 2500 BC, noting at the end no treatment options available for this disease (Tauxe, 2015; Zink et al., 1999). Therefore, it would be not an overstatement to say that if cancer has accompanied us throughout our evolutionarily development, the anti-cancer protective mechanisms likely have occurred and evolved alongside.

As live organisms are the products of Darwinian evolution, cancer is believed to be the product of the same process, just in the microscale and vastly accelerated. In a similar fashion as "The Selfish Gene" hypothesis, proposed by Richard Dawkins in his famous book from 1976, cancer development can be seen as the effect of a "selfish cell" – meaningless "replicator". Evolutionary selective advantage can be defined by the genetical fitness, which is the degree of the species/cell capabilities to succeed in the certain environment. Upon shifts in the environment, the fitness of a species changes, pushing for more evolutionary adaptations. Cancer cells do not only adapt to the environment they evolve within, they actively shape it in their own favour. Hence, the more time they have, the worst for the host. Every day healthy tissues are exposed to damage caused by external stressors (UV, toxins) but also internal - as the result of their own metabolism and cell bio-machinery. If the cell is unable to fix the damage, it must be eliminated for the sake and benefit of the whole organism. Using specialised self-defence mechanisms like immune system, hundreds of dangerous, pre-malignant cells, are being efficiently eliminated before they pose any threat to the well-being of the body.

Just from these basic rules of biology we can imagine the optimal anti-cancer strategy – reduce the toxic exposure, strengthen your body defence, keep your organism environment hostile for malignant cells, and if it occurs, the sooner you find it the highest chances you have to stop it.

I. Diet

Diet is arguably one of the most influential environmental factor in human health and well-being. Moreover, it has profound cultural and psychological impact on people's life, emerging from the inevitable dependency of human existence on the food supply during centuries. Despite the enormous complexity of the relation between food and health, people very early noticed that what they are eating impacts their body. Hence, throughout time dietary "common knowledge'' has been shaped and evolved alongside human civilization. Nowadays, scientists involved in nutrition research have an uneasy task to put aside these common beliefs and accurately investigate the relation between food and health, followed by reasonable interpretation and hopefully practical implications of obtained results.

1. Environment is the primary factor in cancer prevention

For a long period of time, mainly because of the excitement surrounding genetic code discovery, the scientific interest in studying the impact of environmental factors on human cancer diseases was put aside. Indeed, there have been many studies investigating the familial genetic components and the susceptibility of developing particular type of cancers, with spectacular cases of specific gene(s) identification that was primarily responsible, like the famous onco-suppressor *Breast Cancer 1* gene (*BRCA1*) which when found mutated indicates higher chances of developing breast and ovarian cancer (J. M. Hall et al., 1990; Miki et al., 1994). Despite this initial success, very soon it become clear that a majority of cancer cases cannot be attributed to genetic variations, and the biology is much more complex than it was assumed.

First scientific proofs of the dominant role of the environment in cancer incidents came from observational studies investigating the health of the people that migrated from their country of origin to another country, with different environment and different risk of development of particular disease. With time and generations, immigrants acquired similar cancer incidents rates as the endogenous population they shared the new environment with. For example, Japanese people that are characterised by relatively high stomach and low prostate cancer incidents, after migrating

to Hawaii started to exhibit higher rates of prostate cancer and lower rates of stomach cancer, statistically resembling the native Hawaiian population (Stemmermann, Nomura, Chyou, Kato, & Kuroishi, 1991). Another evidence come from studies on monozygotic and dizygotic twins, where monozygotic twins shares 100% of genetic material, and dizygotic twins are sharing statistically 50%, which allows with the statistical power to distinguish the contribution of genes and the environment to the physical and health outcome. In that case it was also concluded that the overwhelming contributor of cancer development was the environment (Lichtenstein et al., 2000).

It is now commonly accepted that as few as 5-10% of all cancers can be linked to the heritable genetic background (Anand et al., 2008; Lichtenstein et al., 2000).

2. Environmental contributors in cancer development

A. Epidemiological approach of diet and cancer connection

The environment consists of many interconnected elements, including exposure to air, sun, natural radiation, infections – that might not be easily modifiable, and the factors that are to some extend the result of the personal choice – diet, physical activity, alcohol consumption, social interactions. Scientific interest in the diet and health connection appeared in the 19th century, but real evaluation had to wait until any reliable populational data were collected to perform the analysis. Exploring the history from the beginning of the twentieth century, it has been said at the foundation of American Cancer Society in 1913 by Frederick Hoffmann that "nutritional influences on the induction of cancer [have to] be analysed'' (Campbell, 2017).

One of the first attempts to evaluate the impact of the many factors on risk of cancer development have been published by Richard Doll and Richard Peto in 1981 at the Oxford University (Doll & Peto, 1981). They listed tobacco as the first single preventable cause of cancer accounted for 30% of all cancer cell deaths in 1978 (**Figure 1**). Another major factor in their analysis was diet (including related overnutrition) that with great uncertainty was assessed to contribute to 35% (10-70% range) of cancer incidents. Interestingly, already at that time the authors have noticed that the impact of diet can vary dramatically between cancer types, pointing to stomach and colorectal cancer as the most affected by nutrition. Although in the following years there was fair amount of criticism of the Doll and Peto findings, their estimations based on 1979 data collection hold to be true after three decades of additional research and have been largely consistent with recent estimations (**Figure 1**) (Blot & Tarone, 2015; Colditz, Sellers, & Trapido, 2006; Doll & Peto, 1981; Song & Giovannucci, 2015b). The important difference is that nowadays researchers separate

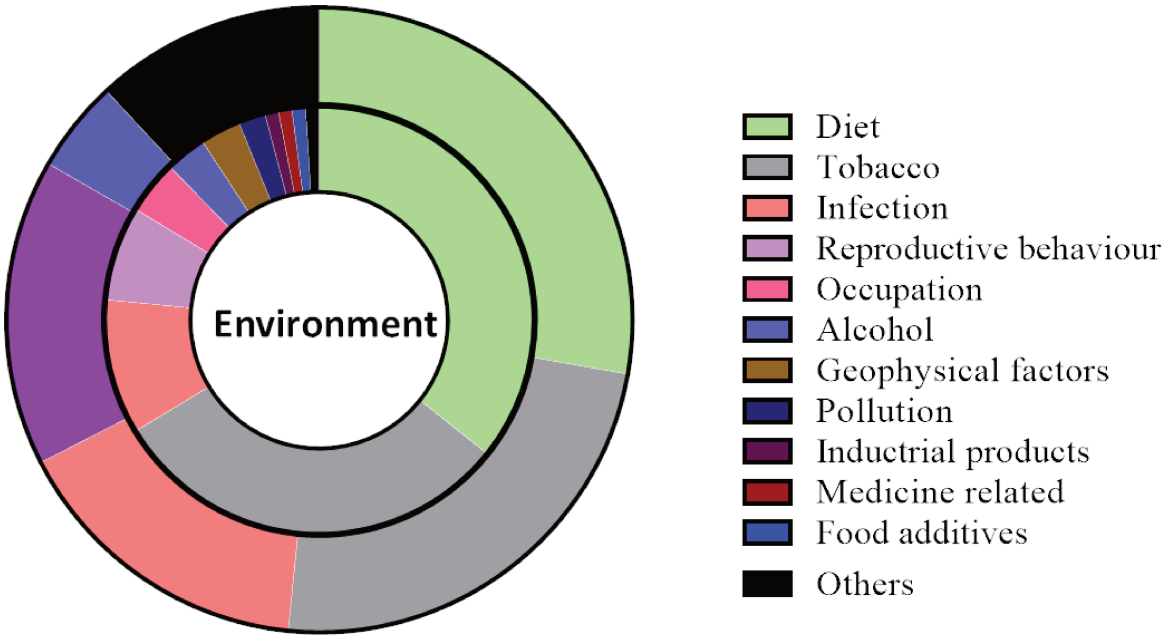


Figure 1: Environmental cancer risk factors

Environmental contribution to cancer deaths in United States that could be attributed to specific factors and avoided by lifestyle changes in each category. The inner circle is a representation of factors as estimated by Doll and Peto in 1981, and the outer circle is the re-evaluation of their findings by Anands in 2008. The graph does not represent the predicted uncertainty of a given estimation, which in some cases was relatively high (see comments in the main text).

overnutrition (being obese and overweight) from the ‘‘pure’’ dietary impact, statistically disconnecting these two elements. In addition, it is very difficult to separate the impact of the early life nutrition (prenatal and early postnatal) which can have long lasting effects on the individual health (Song & Giovannucci, 2015b).

Taken together, in spite of exponential increase in the number of publications in the field of cancer epidemiology, the initial findings and estimations of Doll and Peto remain largely valid and inspired many clinicians and researchers to investigate the impact of nutrition on cancer development in the followed years (Campbell, 2017).

B. Modern methods of evaluating diet-cancer relation

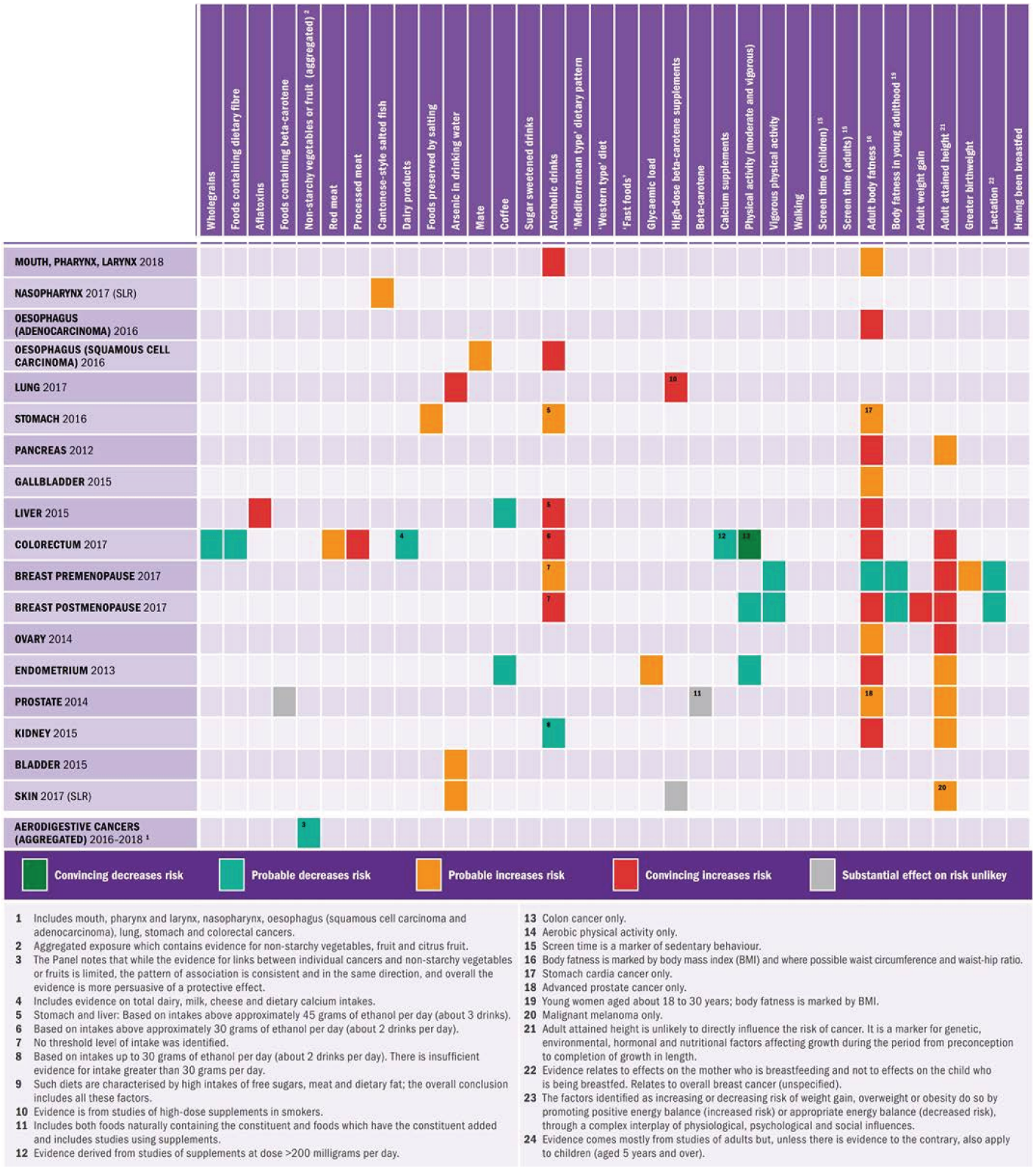
The scientific evidence comes from various types of research, stretched on the spectrum from retrospective epidemiology studies (mainly correlation studies) and more accurate clinical dietary trials that are much more difficult to conduct and possess serious limitations (i.e. sample size and number of factors being evaluated at the same time). In laboratory research, numerous animals and cell culture models have been developed and tested in promise to generate accurate biological hypothesis and then translate them into more complex experimental setups. Regardless the fact that in a majority of cases conclusions based on these models cannot be directly translated to the human dietary recommendations, they have been very helpful in process of understanding the basic cell biology and metabolism. They have also been an inspiration to pursue many hypothesis grounded on their initial results in more complex and clinically more relevant experiments and trials, which provided further understanding and ideas on the studied subject.

Combining studies across those different levels of scientific research allows to formulate reasonable scientific-based dietary recommendations for the general population, with acknowledged degree of certainty based on existing evidence concerning each recommendation in relation to specific cancer type (**Figure 2**). This requires a tremendous amount of work combining the results from various types of research, composed of various scientific approach and heterogenous sample size. The scientific accuracy of the results from each study has to be assessed by the independent panel of experts to adjust the weight of the evidence in relation to all other studies. This process is to be repeated and the conclusions updated as every new research brings

more data. One of the biggest and systematically revised evaluations comes from The Continuous Update Project (CUP), established by the effort of The American Institute for Cancer Research and The World Cancer Research Fund International (AICR/WCRF) in 2008 (Bandera et al., 2016). As part of the CUP, all scientific research currently available is collated and added to a database and systematically reviewed by a team at Imperial College of London, evaluated by an independent panel of experts who ultimately interprets the evidence to make conclusions based on the existing body of scientific evidence.

As a result of this simultaneous comparative analysis of various datasets, it is now evident that different cancer types are differentially associated with dietary patterns, with some of them more prone to have diet-modifiable outcomes than the others. Hence, colorectal cancer incidence and progression appears stronger affected by dietary factors than the other cancer types, which is very logical taking into consideration the direct contact that colon tissue has with the digestion of food. There are very few recommendations that have been shown to affect personal risk of particular cancer development with strong scientific evidence (**Figure 2**). For example, processed meats and alcohol consumption increase risk of developing colorectal cancer, whereas consumption of whole grains, dietary fibre containing foods and dairy products decreases that risk (Gonzalez & Riboli, 2010; Vieira et al., 2017).

On the other hand, positive impact of any of the food group impacting cancer risk cannot be easily attributed to some specific nutrients of this food and replaced by supplementation. For example, consumption of foods rich in carotenoids that has been convincingly shown to have an anticancer effects and thought to be cancer protective, did not turn out to have beneficial effect on lung cancer prevention and progression in the form of beta-carotene supplementation. Instead, beta-carotene supplementation resulted in increased risk of developing lung cancer in current and former smokers (Druesne-Pecollo et al., 2010). The other interesting observation coming from extensive effect of research on milk products is that dairy consumption increases prostate cancer risk, but at the same time decreases breast and colorectal cancer risk (Aune et al., 2015; Gonzalez & Riboli, 2010; Vieira et al., 2017; M. Yang et al., 2015).



1 Includes mouth, pharynx and larynx, nasopharynx, oesophagus (squamous cell carcinoma and adenocarcinoma), lung, stomach and colorectal cancers.
2 Aggregated exposure which contains evidence for non-starchy vegetables, fruit and citrus fruit.
3 The Panel notes that while the evidence for links between individual cancers and non-starchy vegetables or fruits is limited, the pattern of association is consistent and in the same direction, and overall the evidence is more persuasive of a protective effect.
4 Includes evidence on total dairy, milk, cheese and dietary calcium intakes.
5 Stomach and liver: Based on intakes above approximately 45 grams of ethanol per day (about 3 drinks).
6 Based on intakes above approximately 30 grams of ethanol per day (about 2 drinks per day).
7 No threshold level of intake was identified.
8 Based on intakes up to 30 grams of ethanol per day (about 2 drinks per day). There is insufficient evidence for intake greater than 30 grams per day.
9 Such diets are characterised by high intakes of free sugars, meat and dietary fat; the overall conclusion includes all these factors.
10 Evidence is from studies of high-dose supplements in smokers.
11 Includes both foods naturally containing the constituent and foods which have the constituent added and includes studies using supplements.
12 Evidence derived from studies of supplements at dose >200 milligrams per day.
13 Colon cancer only.
14 Aerobic physical activity only.
15 Screen time is a marker of sedentary behaviour.
16 Body fatness is marked by body mass index (BMI) and where possible waist circumference and waist-hip ratio.
17 Stomach cardia cancer only.
18 Advanced prostate cancer only.
19 Young women aged about 18 to 30 years; body fatness is marked by BMI.
20 Malignant melanoma only.
21 Adult attained height is unlikely to directly influence the risk of cancer. It is a marker for genetic, environmental, hormonal and nutritional factors affecting growth during the period from preconception to completion of growth in length.
22 Evidence relates to effects on the mother who is breastfeeding and not to effects on the child who is being breastfed. Relates to overall breast cancer (unspecified).
23 The factors identified as increasing or decreasing risk of weight gain, overweight or obesity do so by promoting positive energy balance (increased risk) or appropriate energy balance (decreased risk), through a complex interplay of physiological, psychological and social influences.
24 Evidence comes mostly from studies of adults but, unless there is evidence to the contrary, also apply to children (aged 5 years and over).

Figure 2: Summary of strong evidence on diet, nutrition, physical activity in cancer prevention.

Table summary of strong evidence on nutrition and preventiveness of specific cancer types according to World Cancer Research Fund/American Institute for Cancer Research evaluated in the Continuous Update Project (CUP) Expert Report from 2018. Details are available at dietandcancerreport.org

Yet, epidemiological studies cannot provide the evidence that isolated nutrients (like vitamins, minerals and phyto-compounds) can represent an independent factors for cancer risk (Baena Ruiz & Salinas Hernandez, 2014).

The dietary guideline recommendations are designed for the healthy adult population, and any single conclusion within them does not represent a recommendation on its own, but forms a part of the overall "body of evidence". Because it is already difficult to draw decisive conclusions regarding healthy individuals, it is even more challenging to design recommendations for patients that have been already diagnosed with cancer. There is limited amount of evidence on the dietary impact and it is very much affected by the type of cancer and received treatment combined with side effects (Robien, Demark-Wahnefried, & Rock, 2011). It is very likely that with the improvement in the experimental methods for gathering and analysing dietary patterns and disease our knowledge on the subject of nutrition will improve substantially in the near future, especially with the introduction of new technologies. In addition, the use of "big data" from large patient databases and human samples collection coupled with multivariant statistical analysis will allow the researchers to more accurately point out the cause and effect, and reduce the impact of unrelated factors. **Therefore, the experimental results and dietary regimens described in the follow up chapters will be based entirely on experimental studies done on animal and *in vitro* cell culture models, hence the hypothesis and any conclusions draw from these studies cannot be extended and are not directly applicable for human nutrition.**

Even though the official recommendations are scientifically cautious, modest and designed to be "achievable" by the majority of the population, a surprisingly low number of people follows even the minimal requirements for healthy lifestyle (Krebs-Smith, Guenther, Subar, Kirkpatrick, & Dodd, 2010; Moore et al., 2015). For example, despite the numerous advertisement and political campaigns promoting fruits and vegetables consumption, most American fails to meet national recommendations, and only 8% of the population consume enough amounts of vegetables and 14% enough amounts of fruits. This low adherence to the healthy lifestyle is very likely one of the reasons why estimated 50-80% of cancer could be entirely avoided, and although this statistics sounds very miserable, it should be rather taken us a strong incentive and the motivation for change,

as there is evidently a big room for improvement (Anand et al., 2008; Colditz, Wolin, & Gehlert, 2012; Song & Giovannucci, 2015a).

C. Laboratory approaches investigating nutrition

In laboratory studies macro- and micro-nutrient/s modulation has been shown to affect key biological cell processes, for instance regulating the metabolism and cell growth by energy sensing AMPK (Hardie, 2007) pathway or nutrient sensing mTOR (Schmelzle & Hall, 2000).

One of the biggest and most comprehensive dietary study, The European Prospective Investigation into Cancer and Nutrition, found significant associations between serum levels of molecules (markers) involved in key carcinogenesis pathways and risk of developing different forms of cancer (Gonzalez & Riboli, 2010). For instance, high serum concentration of IGF-1, major anabolic hormone produced generally by the liver, was associated with increased risk of prostate cancer, which agrees with the previous experimental studies implying the importance of insulin/IGF-1 pathway in carcinogenesis and tumor progression (Culig et al., 1994; Harvey, Lashinger, Otto, Nunez, & Hursting, 2013; Levine et al., 2014). This and many other biomarkers have been used as substitute to assess and compare the dietary and pharmacological interventions performed in rigours laboratory conditions with the outcomes that we observe in the more complex real life environment. In the following section the most comprehensive studies of regimens found to affect tumor development will be described.

3. Dietary regimens in cancer progression

Even though the first study assessing the effect of diet on cancer was published over 100 years ago, the notion of dietary regimen as health promoting practices has been observed and included in various religious and traditional practices over the history. Although different in details, commonly these practices can be divided and characterised by; 1 - restricted consumption of all food over certain period (fasting regimens); 2- restriction of the specific type of foods (i.e. pork, meat or dairy products); or 3 - requiring specific method of food preparation (i.e. halal, kosher). Some of these dietary practices have been proposed to impact on health issues, including cancer incidence, and

metabolic biomarkers, hence studies have been conducted to test various beliefs with the scientific rigor and in the scientific fashion.

A. Calorie restriction

Caloric restriction (CR) is the reduction of caloric intake generally by 10-40% without the induction of malnutrition. CR is currently the most robust dietary intervention known to increase healthy life (fewer diseases) and prolong lifespan across a spectrum of living organisms, from yeast to mice and primates, and has been under investigation for almost a century (McCay, Crowell, & Maynard, 1989; Meynet & Ricci, 2014; Tannenbaum & Silverstone, 1953). More importantly, CR has increasing amount of evidence supporting its role in inhibiting tumor development, with the first experimental studies described over 50 years ago (Tannenbaum & Silverstone, 1953). In fact, its antitumor effects have been proved to be substantial and spans across various types of spontaneous and inducible cancer models summarised in (**Figure 3)** (Pallavi, Giorgio, & Pelicci, 2012): mammary (Kharazi et al., 1994; H. W. Li, Zhao, & Sarkar, 1994), leukemia (Yoshida, Hirabayashi, Watanabe, Sado, & Inoue, 2006; Yoshida et al., 1997), liver (Ploeger, Manivel, Boatner, & Mashek, 2017), pancreatic (Lashinger et al., 2011; Roebuck, Baumgartner, & MacMillan, 1993), colon (Dirx, van den Brandt, Goldbohm, & Lumey, 2003; Mai et al., 2003), breast (Phoenix, Vumbaca, Fox, Evans, & Claffey, 2010), and prostate (Bonorden et al., 2009). Furthermore, when tested, the highest degree of restriction was associated with the highest level of protection (**Figure 3**) (Kumar, Roy, Tokumo, & Reddy, 1990; Ruggeri, Klurfeld, Kritchevsky, & Furlanetto, 1989). The effect of CR has been largely attributed to the modulation of phosphoinositide 3-kinase (PI3K) and Protein kinase B (PKB), also known as AKT pathway, since tumors carrying a mutation in either PI3K or PTEN (negative regulator of PI3K) genes failed to respond to calorie restricted diets (Kalaany & Sabatini, 2009), hence the modulation of AKT activity seems to be a crucial factor in CR antitumor effects (Curry et al., 2013) (**Figure 4**).

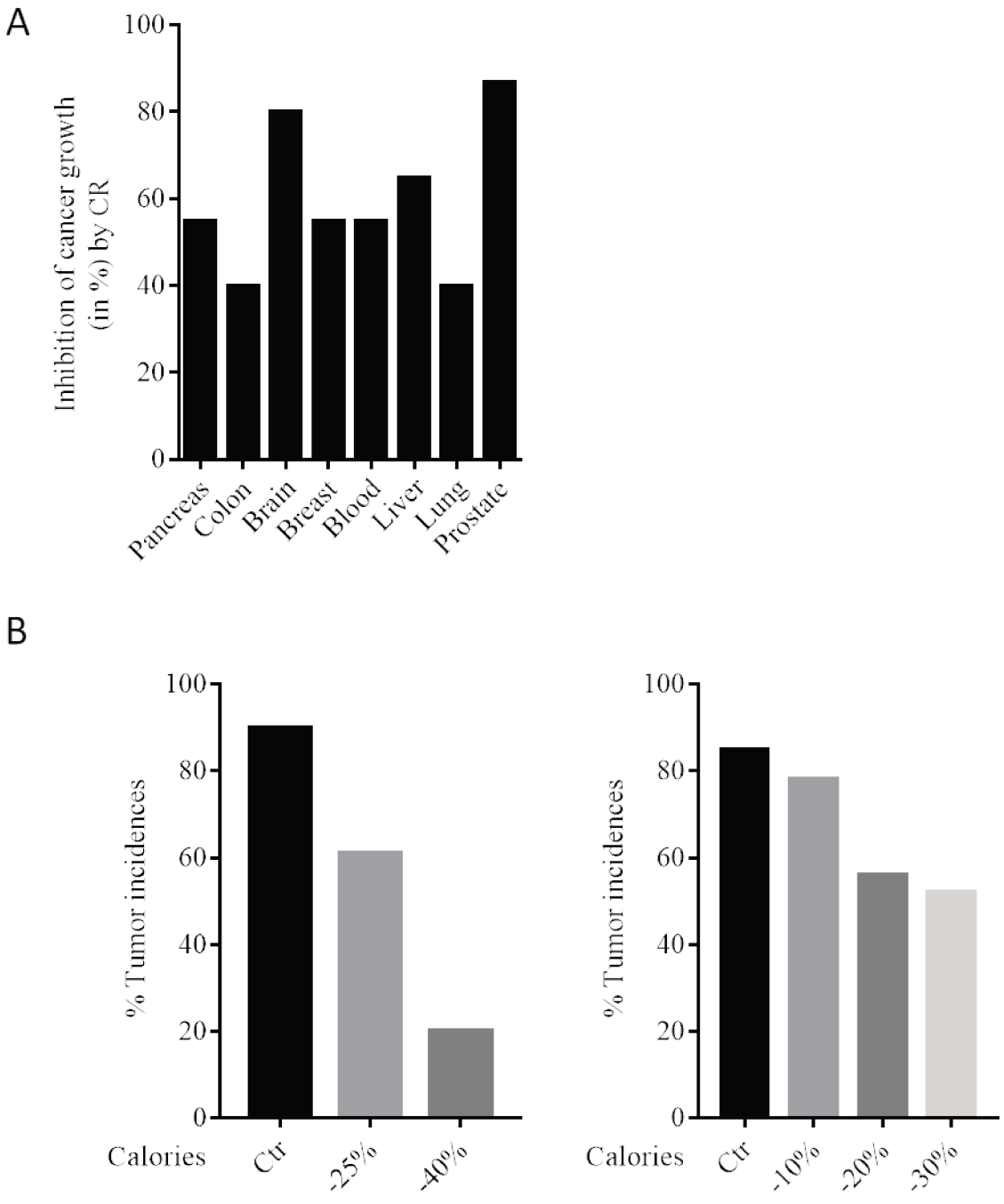


Figure 3: Caloric Restriction and Cancer progression in experimental studies.

Caloric Restriction and Cancer. (A) CR effectively inhibits cancer growth of a variety of cancer models as summarised and presented by Pallavi and Giorgio et al., 2012. (B) CR effect is dose dependent (reduction dependent) as cancer protection is proportionally higher with calories intake reduction in azoxymethane-induced colorectal tumors in F344 rats – graph on the left (Kumar et al., 1990), and in DMBA-induced mammary tumors in Sprague-Dawley rats – graph on the right (Ruggeri et al., 1989).

Insulin like growth factor I (IGF-1) is primarily synthesised and secreted by the liver upon growth hormone (GH) stimulation. Circulating levels of IGF-1 and IGF-1 binding proteins have been strongly correlated with risk of developing various cancers (Anisimov & Bartke, 2013; Cao et al., 2012; Crowe et al., 2009; Endogenous et al., 2010). Striking evidence of IGF-1 role in cancer induction comes from individuals who carry inheritable mutations in the growth hormone receptor (GHR) gene, which disables liver centred production and secretion of IGF-1 (Guevara-Aguirre et al., 2011). Compared to their non-mutation bearing relatives, these individuals are almost completely protected against development and death from cancer.

On the basis of the above findings, the scientific rush to discover signaling molecules and compounds mimicking CR has started (**Figure 4** and **Table 1**). In the context of cancer, main interest focused on mammalian Target of Rapamycin (mTOR), AMP-activated protein kinase (AMPK), family of sirtuins and more recently autophagy pathways.

- **mTOR**

The central hub of metabolic cell processes coordination and nutrient availability is focused on mTOR pathway. The history of mTOR discovery is one of the example how the broad range of clinical and observational insights could lead to the most scrupulous experimental investigations in very simple yeast organisms, and then it extended its importance throughout all of the phylogenetical kingdoms (Manning, 2017; Sabatini, 2017).

mTOR owes its name to the natural inhibitor of its activity found in the bacteria isolated from soil on pacific islands the Rapa Nui in 1972, known more widely as the Easter Islands. The isolated bacteria strain *Streptomyces hygroscopicus* has been found to be a source of variety of biologically relevant molecules like Sirolimus (another name for rapamycin), ascomycin (immunosuppressant), nigericin (antibiotic), milbemycin (antiparasitic agent). Sirolimus has been clinically used for many years as an immunosuppressive drug, even before knowing its actual mechanism of action.

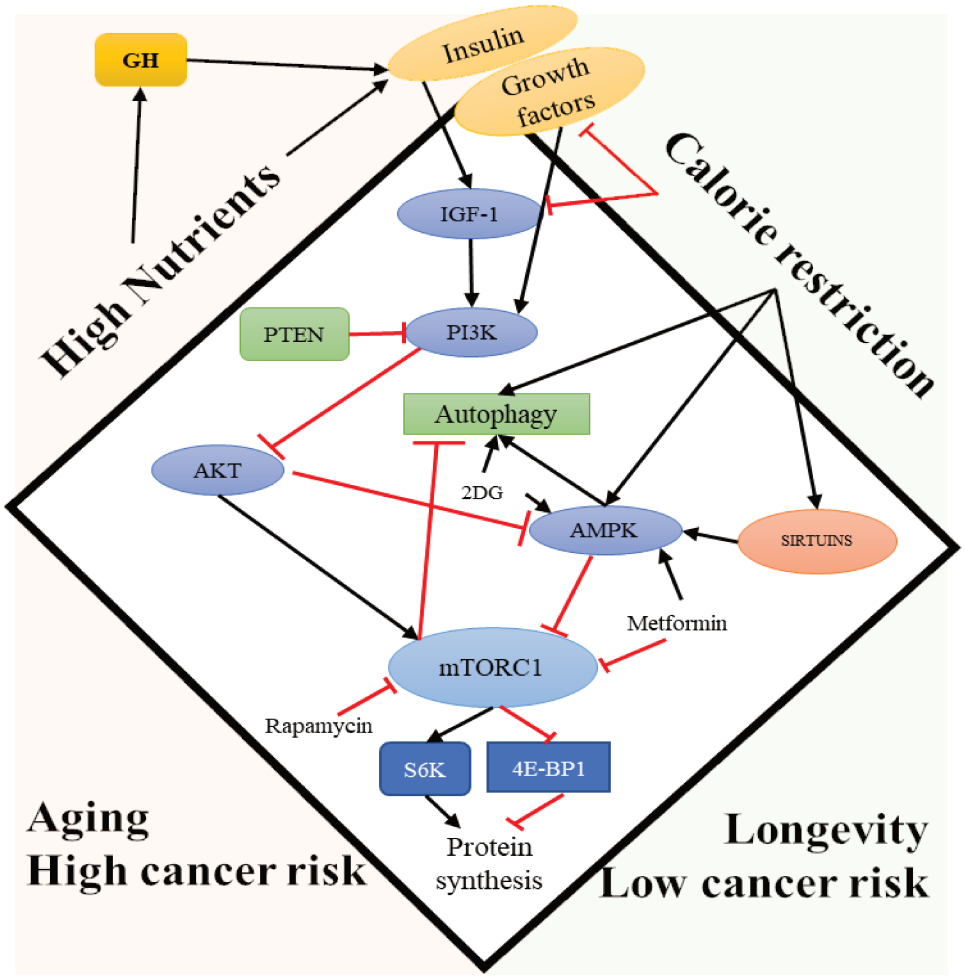


Figure 4: Molecular pathways implicated in Calorie Restriction

The simplified scheme of major pathways involved in nutrient and growth factor sensing in nutrient rich and CR conditions on cancer risk and longevity. Nutrients, insulin and growth factors activates IGF-1 and the mTOR pathways, promoting aging and cancer risk, whereas CR activates AMPK and sirtuins pathway which increase longevity and reduce cancer risk. The mechanisms of action of calorie restriction mimetics – 2-deoxyglucose (2DG), metformin and rapamycin are indicated. IGF-1- insuling-like growth factor 1; AMPK-AMP-activated protein kinase; PI3K-phosphoinositide 3-kinase; mTORC1 – mammalian Target of Rapamycin complex 1; S6K-Ribosomal protein S6 kinase beta-1; 4E-BP1- Eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1; GH- growth hormone.

By comparison with another bacterium derived immunosuppressive drug tacrolimus, which shares with rapamycin the same chemical binding domain to peptidyl-prolyl isomerase FKBP12, but

produces distinct effects on T cell signaling, the existence of another target of rapamycin was speculated and finally described (Heitman, Movva, & Hall, 1991).

Now we know of existence of two functional complexes mTORC1 and mTORC2 in mammals, with mTORC1 being the main target of rapamycin and currently is much more extensively studied compared to mTORC2. Therefore, mTORC1 will be referred as mTOR from now on.

mTOR in known as a master growth and metabolism regulator that senses and integrates diverse nutritional and environmental cues such as growth factors, cellular energy levels, stress and amino acids availability. It exert its actions through control of main cell anabolic and catabolic processes including mRNA translation, lipid synthesis and autophagy (Yecies & Manning, 2011).

The small GTPase Ras homolog enriched in brain Rheb (GTP-bound form) is a major activator of mTOR. Rheb is negatively regulated by the tuberous sclerosis heterodimer TSC1/2, which converts Rheb to its GDP-bound form. Most upstream signals are channelled through AKT and TSC1/2 to regulate the GDP/GTP state of Rheb (Huang & Manning, 2009). In contrast, amino acids can activate mTOR independently of TSC1/2 or AKT axis, acting by the spatial translocation of mTORC1 to the lysosomal surface where it is activated upon interaction with Rheb (Sancak et al., 2010).

Recently, mTORC1 has been shown to be responsive to changes in purine nucleotides pool in a similar manner to its sensing of amino acid pool (Hoxhaj et al., 2017). In comparison, amino-acid sensing is orchestrated by the GATOR1-Rag branch and intracellular nucleotide levels seems to be dependent on the TSC-Rheb axis (Emmanuel et al., 2017; Hoxhaj et al., 2017). This recently described nucleotide sensing property of mTOR is already under investigation as metabolic target in cancer treatment and has been shown to provide vulnerabilities of cancer cells to further therapy, such as use of nucleotide synthesis IMPDH1/2 enzyme inhibition (Valvezan et al., 2017). mTOR driven nucleotide biosynthesis has been found as an important factor in cancer development, as tumor cells requires significantly more pyrimidines than can be supplied by extracellular import (Howell, Ricoult, Ben-Sahra, & Manning, 2013; Moyer, Oliver, & Handschumacher, 1981). Thus, based on these findings we can expect novel strategies to target metabolic vulnerabilities in cancer treatment.

Cellular stress or energy deficiency can inhibit mTOR activity. AMPK activation in response to low energy levels can lead to TSC2 phosphorylation and mTOR inhibition. In addition, AMPK seems to directly exert its action on mTOR activity by phosphorylation of protein Raptor, one of the members of the mTORC1 complex (Gwinn et al., 2008).

The approved uses and ongoing testing of various mTOR inhibitors (sirolimus and Rapalogues, rapamycin derivatives) in the clinic for diseases such as tuberous sclerosis complex (TSC mutated genetic disorder, characterized by disseminated growth of benign tumors), illustrate the importance of mTORC1 signaling in cancer growth.

Since its discovery, the modulation of mTOR activity has been believed to hold the promise to slow down and/or improve many negative outcomes attributed to aging process, for example reducing the risk of age-related disorders, which will be discussed further in the next sections (Johnson, Rabinovitch, & Kaeberlein, 2013).

- **AMPK**

5' adenosine monophosphate-activated protein kinase (AMPK) is a cell energy sensor responsible to maintain ATP levels stable over energy crisis, signed by drop in the ratio of AMP/ATP, exerting its action through shutting down anabolic processes and promoting catabolic machinery. For example, AMPK can inhibit energetically demanding protein synthesis by activation of eukaryotic elongation factor 2 kinase (eEF2K) (Leprivier et al., 2013).

As in the case of many others, AMPK signaling evolved mechanisms to protect cells from stress and enable survival over periods of energetical scarcity. The activation of AMPK leads to inhibition of mTOR, therefore under some conditions it slows down growth and tumor progression, but at the same time it can lead to tumor cell survival by induction of protective autophagy during metabolic/oxidative stress, potentially interfering with some of the chemotherapeutical treatments (Faubert et al., 2013; Jeon, Chandel, & Hay, 2012). Hence, the duration and magnitude of AMPK activity, as well as the timing of therapeutic intervention targeting AMPK pathways seem to be crucial in harnessing its antitumor effects.

In syngeneic mouse model of MYC-driven lymphoma activation of AMPK by low-carbohydrate diet feeding resulted in sensitization of cancer cells to ABT-737-induced cell death and extended mice survival. Mechanistically, low-carbohydrate diet reduced serum glycemia, which in turn activated AMPK and inhibited mTOR activity, leading to downregulation of myeloid cell leukemia-1 protein (MCL-1), key anti-apoptotic protein contributing to ABT-737 resistance (Rubio-Patino et al., 2016).

There are several compounds found to directly or indirectly activate AMPK, and many of them have been investigated as dietary restriction mimetic, like metformin, resveratrol, quercetin, genistein, berberine, curcumin- indirect, and AICAR and salicylate as direct AMPK inhibitors (J. Kim, Yang, Kim, Kim, & Ha, 2016).

- **Sirtuins**

Sirtuins are a family of NAD+-dependent enzymes that modulate the metabolic status of cells through targeting and modifying activity of proteins involved in a wide range of cellular processes, having a key roles in physiology of healthy and malignant cells (Chalkiadaki & Guarente, 2015). In mammals seven sirtuins have been described so far, exhibiting range of functions and cellular localisations (Guarente, 2013). It has been proposed that sirtuins ca serves as sensors of cellular nutrient availability, and their activity modulation can mimic the effects of CR on carcinogenesis (Chalkiadaki & Guarente, 2015). In mammals, CR leads to SIRT1 activation in diverse cells and tissues, that is attenuated by IGF-1 (Cohen et al., 2004). In addition, transgenic mice with SIRT1 overexpression phenotypically mimic mice under CR: they are leaner; with improved glucose tolerance; decreased blood cholesterol and insulin levels; reduced incidence of spontaneous carcinomas/sarcomas, and protection against colon cancer (Bordone et al., 2007; Firestein et al., 2008; Herranz et al., 2010). Despite such promising metabolic effects, SIRT1 induction alone or combined with CR appears to have limited role in cancer protection and do not bring additional benefits, possibly owing to its differential tissue-specific expression and regulation (Boutant et al., 2016; Herranz, Iglesias, Munoz-Martin, & Serrano, 2011).

B. Fasting

Fasting is the practice of withdrawal consumption of any food or its dramatic restriction for a certain period (not particularly defined and specie-dependent). Historically, it might be the oldest and most widely adapted way of dietary regimen among human race, commonly having a religious purpose. Despite existence of anecdotal evidence attributing fasting anti-cancer properties, scientific investigation of the use of fasting-based regimens in cancer prevention and treatment are relatively recent.

Fasting and CR are simultaneously similar in some features but profoundly different in others, and they cannot be treated as the same regimen but with different magnitude. Exercise training could serve as an analogy in order to compare the different outcomes that these two dietary regimens could displayed. As it is commonly known acute/intense high load exercise (fasting) produces differential metabolic and physiological response compared to chronic/low intensity training (CR), which results in gain of higher overall body endurance. As such, CR and fasting can result in different stress response of the organism. Additionally, fasting leads to depletion of glycogen storages and utilisation of ketone bodies as a source of energy, metabolic processes which have not been reported in CR.

The first days of fasting display high rate of gluconeogenesis and amino acids being catabolized and used as the source of energy, especially alanine coming probably from muscles. Insulin is decreased, as oppose to increase in glucagon. As fasting continues, the body starts to use its fat deposits for energy production which results in progressive ketosis, which means the increase of ketone bodies produced by the liver and utilized by peripheral tissues as a fuel, including acetoacetate and β-hydroxybutyrate (Kerndt, Naughton, Driscoll, & Loxterkamp, 1982). Ketosis, state previously known mainly from its induction in diabetic individuals as an effect of insulin insensitivity or abrogated production, is a natural condition that the body resorts to survive such nutrient starvation.

Mechanistically, fasting results in the inactivation of mTOR and Ras-PKA and activation of Rim15, a key enzyme indispensable for some of the fasting related beneficial outcomes. The last

event produces increased expression of proteotoxic and oxidative stress response genes, participating in the induction of the fasting-protective effects (Wei et al., 2008). T

The protective cellular effects have also been attributed to the decrease in body general inflammation under CR or food deprivation. Interestingly it has been reported that level of β-hydroxybutyrate specifically (one of the ketone bodies) could be responsible for inhibition of inflammasome by suppression of NLRP3-dependent inflammasome activation (Youm et al., 2015).

Very interesting phenomena associated with fasting are Differential-Stress-Resistance (DSR) and Differential-Stress-Sensitization (DSS) (Buono & Longo, 2018). Those refer to the differential effect of fasting-induced stress between healthy and malignant cells, leading to higher and lower resistance respectively to additional stressors (like chemotherapy) (**Figure 5**). This surprising effect could simultaneously lead to higher cytotoxicity of anti-cancer targeting cancer cells, at the same time having lower side-effects and toxicity towards healthy tissues at the same time. For example, 48-72h period of fasting protects mice from otherwise deadly doses of doxorubicin and etoposide (C. Lee et al., 2010). In addition, 72h fasting period mice had lost roughly 20% of their initial body weight, reduced glucose levels by 41% and IGF-1 levels by 70%. This protection was lost under restoration of circulating IGF-1 levels with simultaneous injection during the fasting period. In contrast, malignant cells are being more prone to cytotoxic effect under fasting conditions (D'Aronzo et al., 2015; C. Lee et al., 2012; Shi et al., 2012). For example, 24h fasting synergistically potentiated gemcitabine effectiveness in a pancreatic cancer xenograft model (D'Aronzo et al., 2015). The explanation of this paradoxical phenomenon may lay on the constant activation of oncogenes in tumor cells, that not able to be switched off pushes the cell to grow and proliferate even against the worsening of extracellular conditions, inherently making them more susceptible to anti-cancer treatment. On the other hand, healthy cells are able to sense nutrient scarcity by responding to extracellular signals (GH, IGF-1), shut down metabolism and prepare their survival mechanisms in order to thrive.

Currently it is not clear how long human individual should fast to obtain metabolic or cancer associated benefits with this type of regimen. Although there is compelling evidence defining duration and types of fasting regimen in cancer mouse models (24-48h, intermittent fasting, fasting mimicking diet), mouse metabolism and evolutionary adaptation mechanisms for starvation profoundly differ from those in humans. Limited experiments done in clinics indicate that discrepancy. For example, 72h but not 24h fasting provided a reduction in leukocyte DNA damage accompanied with less side effects in patients receiving chemotherapy (Buono & Longo, 2018).

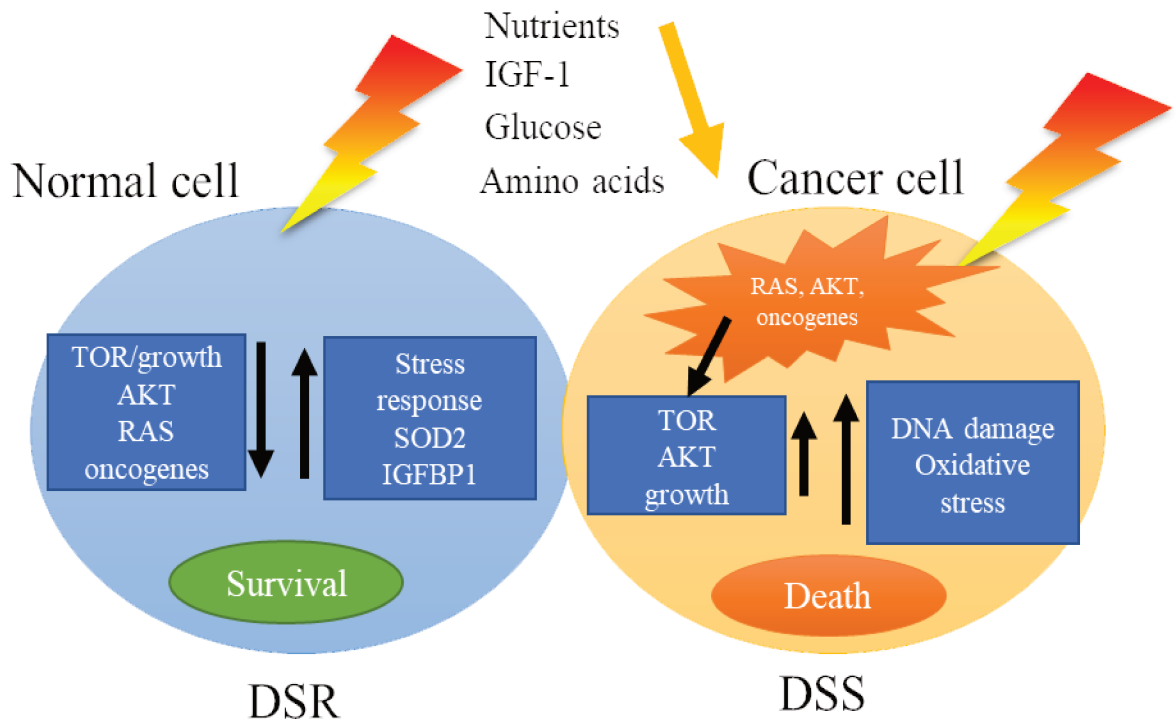


Figure 5: Differential-Stress-Resistance (DSR) and Differential-Stress-Sensitization (DSS) phenomena.

Fasting effect on Differential-Stress-Resistance (DSR) and Differential-Stress-Sensitization (DSS) as proposed by Buono and Longo 2018. Extracellular stress as an effect of short-term fasting result in opposite effects on normal versus malignant cell. As opposite to cancerous cell, healthy cell can adapt to acute macronutrient shortages by downregulating its metabolism and inducing stress response pathways, whereas oncogene driven metabolism of cancer cell hampers its adaptational mechanisms and sensitize it towards chemotherapy. As a result, upon fasting conditions healthy cells are more resistant and malignant cells more sensitized towards additional treatment. IGF-1- insulin-like growth factor 1; TOR- Target of Rapamycin; IGFBP1- insulin-like growth factor binding protein 1.

Currently, it has been shown in a pilot study that 48h fasting prior to chemotherapy is safe and might provide beneficial effects reducing DNA damage in healthy cells such as lymphocytes and myeloid cells (Bauersfeld et al., 2018; de Groot et al., 2015).

Interestingly, in a recent study evaluating the benefits of CR and fasting in male C57BL/6J mice, a single daily meal (12-13h fasting) and 30% CR resulted in higher mouse survival, lower insulin resistant and fasting blood glucose levels; and delayed onset of liver pathologies as compared with *ab libitum* diet regardless diet composition (Mitchell et al., 2018). These authors tested two different diets, one of them with higher fructose (but equal percentage of carbohydrates) and lower protein. Modulation of macronutrients in both diets did not make any difference in the positive effects of CR and fasting which points out that feeding regimens could have a higher impact on health and lifespan than diet composition *per se*. Importantly, fasting for 12-13h apart of mimicking CR outcomes, did not lead to body weight loss, advantage that should be considered in cancer research.

Nevertheless, the pre-clinical trials of this type of regimens are being currently evaluated worldwide, and likely to shed some light on the potential benefits and limitations. Some of the particularly interested are listed below.

The safety and metabolic outcomes in cancer patients receiving fasting mimicking diet (FMD) - a plant-based, calorie-restricted, low carbohydrate, low-protein, is being evaluated (NCT03340935).

Effects of fasting (36h before and 24h after chemotherapy) on patients with advanced metastatic prostate cancer are also under investigation (NCT02710721). The long term 21-day fasting-like diet (providing only 5% of baseline calories) will examine the changes of disease-associated metabolic biomarkers (NCT03193177).

C. Macronutrient modulation

Caloric restriction in experimental setup relies on restricting 20-40% of general food intake in the treated group as compared to the control. As the food in the control group (not CR group) consists of the complex distribution of macronutrients (fat, carbohydrates and protein) and micronutrients (minerals, vitamins, phytocompounds etc.) with different ratio of these nutrients across the

scientific literature, this adds another level of complexity in order to compare results from different studies and define which nutrient restriction exactly (of the above-mentioned) could be responsible for the CR effect. This problem has been acknowledged at the very beginning of the experimental CR studies at the first half of twentieth century and since it has been debated and generates scientific confusion (Speakman, Mitchell, & Mazidi, 2016).

The nutritional requirement of the organism on each nutrient depends on variety of factors, including stage of body development, age, energy expenditures, genetics, and obviously the species the experiment is based on. Firstly, it is evident that different species will have differential nutrient requirements. Secondly, the nutrient requirements greatly differ along the lifespan, and the body response to macronutrient modulation is different in young than in adult and advanced aged animals. Finally, due to interactions occurring between nutrients and other dietary constituents as well as the non-linearity of responses to many nutrients it creates complexity of the impossible to resolve scale. For example, some vitamins are known as being important in mineral absorption in the guts, therefore their availability can in long-terms manifest as mineral deficiency. As the CR studies have been restricting all of the above nutrients at once, it is rational to assume that the availability of some could fall under nutritional requirement threshold whereas the amount of the other nutrients would still be sufficient, hence the observed effect could be not due to calorie restriction *per se*, but to some nutrients being restricted. For example, 40% CR leads to reduction of 40% calories coming equally from carbohydrates, proteins and fats, but the restriction of one of them can have stronger impact than the others. Therefore, the same nutrient modification can have very different effect on health, longevity and carcinogenesis.

The question of what is the limiting element that CR regimen utilises, either calories or any of the macro/micro-nutrient components, is still under investigation. It is actually very likely that CR acts simultaneously through multiple pathways, thus it results in so vast effects on multiple organism levels and diseases, and it will be unlikely to identify one mechanism that explains them all (Speakman et al., 2016).

Recently, the new approach called Geometric Framework (GF), emerged from the progress in mathematical modelling and statistical analysis could help to assess the impact of single factor among the fluctuations in multiple overlapping factors and become a big step forward in our

understanding of nutrition (Simpson et al., 2017). As oppose to the traditional nutritional experiments with “one variable at a time”, where focus is on the modulation of the single dietary component, GF-based experiments rely on the cross modulation of multi-variables simultaneously (nutrients). The effect of single nutrient modulation can be therefore represented graphically as a point over a given time period or as a moving trajectory within an n-dimensional space, where each dimension is a nutrient. The value of such approach proofed its value first in the model of Drosophila (K. P. Lee et al., 2008) and subsequently in mice (Solon-Biet et al., 2014).

Researchers from Sydney undertook a meticulous work testing the effect of 25 diets fed *ad libitum*, varying in dietary energy density, protein, fat, and carbohydrate. The researchers measured the impact of each diet on food intake, cardiometabolic phenotype, and longevity in 858 C57BL/6 male and female mice, in particularly measuring glucose tolerance, blood pressure, plasma levels of insulin, leptin and amino acids, blood lipid and liver function markers. The diets differed greatly in the content of protein (5%–60%), fat (16%–75%), carbohydrate (16%–75%), and energy density (8, 13, or 17 kJ/g). The study primary goals were to determine what drives CR longevity extension by measuring the lifespan and physiological mechanisms of aging, particularly focusing on the activity of mTOR and amino acid modulations. When they have compared the groups of the highest calorie intake to the group of the lowest (30% CR), the last one accounting for the effect of the other macronutrient modulation (like compensatory overfeeding), they have not found the previously reported lifespan extension. Instead, when mis-matched with all the other factors, reduction in protein intake was clearly positively and dose-dependent correlated with mouse lifespan extension, increasing it by approximately 30% as the protein:carbohydrate ratio decreased (Solon-Biet et al., 2014). Inversely, when the ratio increases hepatic mTOR shows activation, which is associated with the elevated levels of circulating branched chain amino acids. Of note, the low protein high carbohydrates diet was strongly associated with improved glucose tolerance, lower body fat and low blood triglycerides and insulin levels (Solon-Biet et al., 2014; Solon-Biet et al., 2015). The researchers confirmed some of their initial results in the follow-up study investigating the metabolic outcomes of 8-week dietary modifications, where the protein restriction generated the metabolic benefits of CR, at the same time not providing additional advantage when the mice where already under low-protein diets (Solon-Biet et al., 2015). It would be of utmost

importance and interest to test and statistically measure the effect of such spectrum of dietary modulation in a similar study on tumor progression and cancer incidence.

The additional data highlighting the benefits of lower protein consumption comes from the corroborative work where researchers collated the result of populational human study using nutritional survey substantiated with IGF-1 serum levels measurements with cellular *in vitro* and *in vivo* mouse models (Levine et al., 2014). The low protein consumption appears to have a protective effect against all-cause and cancer mortality prior to age 66 and correlates with lower circulating IGF-1 levels in humans. In mice, low protein diet (4% of total protein) resulted in slower cancer growth of subcutaneously injected B16 melanoma cells in C57BL/6 mice as compared to high protein diet (18% from total protein), which was correlated with lower free circulating IGF-1 and higher mIGFBP-1. To test the hypothesis that the modulation of IGF-1 serves as the driving force of tumor growth inhibition, the researchers used GHRKO mouse model, where the invalidation of growth hormone receptor disrupts the Growth Hormone/IGF-1 axis. In GHRKO mice tumor growth of B16 wild type (WT) melanoma cells was slower as compared to the WT C57BL/6 mice, indicating that circulating levels of hormones dictates the tumor progression rates. Of note, the 4% content of protein diet resulted in weight loss and growth retardation in mice BALB/c model, which indicates the high magnitude of the above dietary intervention, which might not be successfully achieved and result in negative health effect over long-term in other models or aged mice. In accord with these observation, the effect of low protein diet was beneficial for young mice (3-4 months old) but not necessarily in older mice (10 months old) which reproduced the observation in human population (Levine et al., 2014).

Of note, studies in *Caenorhabditis elegans* and rats indicate that the proteostasis in endoplasmic reticulum is drastically reduced during aging (Ben-Zvi, Miller, & Morimoto, 2009; Gavilan et al., 2009; Naidoo, Ferber, Master, Zhu, & Pack, 2008; Taylor & Dillin, 2013). It would be interesting to investigate whether such physiological modulation is not connected with different outcomes of nutritional intervention between young and older animals. The relation between proteostasis and aging is further discussed in the chapter describing the unfolded protein response (UPR).

In summary, the latest research appears to point toward protein as the main contributor of long-appreciated CR in general health and aging, linking the protein consumption to its effects on

regulation of mTOR and IGF-1 axis. Whether the low-protein dietary regimens could affect cancer progression and occurrence has to be investigated in the future studies.

D. Diet in the pill - CR mimetics

Based on the findings and impact of CR and dietary related nutritional studies, the promise of developing the pharmacological approach instead of food intervention inspired many scientists to look for molecules that could affect the same pathway as CR does. CR mimetics (CRm) are the group of compounds that phenocopies one or several effects of CR regimen. It was proposed that CRm treatment should phenocopy metabolic, hormonal, and physiological effects of CR, activates stress response pathways observed in CR and enhances stress protection, extent longevity and reduce overall aging and age-related diseases (Handschin, 2016; Ingram & Roth, 2011, 2015; Madeo, Pietrocola, Eisenberg, & Kroemer, 2014). Another proposed definition of CRm is the reduction in overall protein acetylation, which in turn would promote cytoprotective autophagy that would be beneficial for cellular and organ function (Madeo et al., 2014).

The screening of CRm is based on the molecule ability to reproduce one or several intermediate physiological effects that have been observed in the CR studies, such as reduction in glucose, insulin and triglyceride circulating levels. Results of ongoing studies identifying potential CRm are published by the NIA Interventions Testing Program, which every year start evaluation of several compounds for their CRm activity, including among the others aspirin, nitroflurbiprofen, 4-OH-alpha-phenyl-N-tert-butyl nitrone (4-OH-PBN), nordihydroguiaretic acid (NDGA) and rapamycin. Several compounds have been recognised as CR mimetics, which are listed and shortly characterised in **Table 1** but only few of them have been demonstrated to impact cancer progression, and here we will discuss only some of them.

Table 1: Calorie restriction mimetics.

Name	Characteristic	Mechanism of action	Ref.
2-Deoxy-D-glucose	Glucose analog, decreases body weight, insulin levels and body temperature. Cardiotoxic	Glycolysis inhibitor	(Ingram & Roth, 2011)
Metformin	Biguanide, anti-diabetic drug, extends lifespan, reduces incidence of age-related diseases (cancer, cardiovascular disease, and chronic kidney disease). Risk of lactic acidosis	Mitochondrial complex I inhibitor and indirect AMPK activator. Enhances sensitivity of insulin receptors, activates genes reducing glucose synthesis and reduces gene expression of enzymes that increase oxidation of fatty acids	(Kawashima et al., 2013; D. L. Smith, Jr. et al., 2010)
Rapamycin	Antibiotic with pro-longevity effect, reduces pathological Amyloid β levels in animal models of neurodegenerative disorders. Immunosuppresor	mTOR inhibitor, upregulation of autophagy to remove damaged or misfolded proteins	(Ingram & Roth, 2011; Nikolai, Pallauf, Huebbe, & Rimbach, 2015)
Resveratrol	Plant polyphenol (grapes) with lifespan-enhancing effect, prevents age-related diseases, increases insulin sensibility, lowers body weight. Anti-tumor actions	Deacetylase (sirtuins) activator, autophagy induction. Inhibition of phosphoinositide 3-kinase (PI3K)	(J. Li, Zhang, Liu, Chen, & Chen, 2017; Nikolai et al., 2015)
Oxaloacetate	Intermediate of the TCA cycle, increases lifespan, lowers fasting glucose levels	Activation of AMPK via an increase in the NAD+/NADH ratio	(Ingram & Roth, 2015)
Lipoic acid	Antioxidant scavenging ROS and recycling of other antioxidants, counteracts age-related disorders	Induction of Uncoupling Protein (UCP) expression leading to decrease of ROS produced by the mitochondrial electron chain	(Nikolai et al., 2015)
Rimonabant	Anti-obesity drug, improves insulin sensibility, lipid profile, and decreases visceral fat accumulation. High levels of psychiatric side effects	Endocannabinoid-1 receptor blocker	(Horn, Bohme, Dietrich, & Koch, 2018)
Hydroxycitrate	Anti-obesity agent, increases autophagic flux, anti-cancer effects	Competitive inhibitor of the ATP citrate lyase (AcCoA depletion), autophagy induction	(Pietrocola et al., 2016)
Spermidine	Natural polyamine, anti-cardiac aging effects, reduces arteriosclerosis, anti-aging effects	Acetyltransferase (EP300) inhibitor, autophagy induction	(Eisenberg et al., 2009; Marino, Pietrocola, Madeo, & Kroemer, 2014; Pietrocola, Castoldi, Markaki, et al., 2018)
N-Glucosamine	Natural amino sugar and dietary supplement, extends life span, lowers blood glucose levels	Glycolysis inhibitor, indirect AMPK activator	(Weimer et al., 2014)
Quercetin	Plant polyphenol, expands lifespan, anti-tumor actions, synergism with other autophagy-inducing plant metabolites (resveratrol)	Sirtuin activation, autophagy induction	(Pallauf & Rimbach, 2013)
Genistein	Plant polyphenol (soybeans), extends lifespan, protects against age-associated degenerative disorders, lowers blood pressure	AMPK activation, autophagy induction	(E. B. Lee et al., 2015)
Curcumin	Plant polyphenol (tumeric), anti-inflammatory, anti-oxidant and cell death-inducing properties, improves obesity-associated comorbidities, anti-cancer properties	Histone acetyltransferase inhibitor, autophagy induction. Modulation of Akt and Erk pathways	(Marcu et al., 2006; Pallauf & Rimbach, 2013; Sarker et al., 2015)

Name	Characteristic	Mechanism of action	Ref.
AICAR	Analog of AMP, prevents fat gain, restores muscle mass by preventing inflammation-associated cachectic muscle wasting	AMPK activator	(D. T. Hall et al., 2018; Ruegsegger, Sevage, Childs, Grigsby, & Booth, 2017)
Salicylate	Plant hydroxybenzoic acid, anti-inflammatory properties, prolongs health span and lifespan, reverses high-fat diet-induced insulin resistance, anti-arteriosclerotic and cancer-preventive effects	Inhibitor of the acetyltransferase EP300 (autophagy induction in heart and liver), NF-kB inhibitor, AMPK activator	(Pietrocola, Castoldi, Markaki, et al., 2018)

- **2-Deoxy-D-glucose**

2-Deoxy-D-glucose (2DG) is a chemical analog of glucose, structurally similar enough to be up-taken by the cells as glucose sharing by the same membrane transporter as glucose and then converted by hexokinase to 2-Deoxy-D-glucose-6-phosphate. Because the latter cannot be further metabolised, its being accumulated within the cell and subsequently result in the block of glycolysis. Cancer cells in general have higher requirements for glucose, as well as they have elevated levels of glucose transporters and hexokinase, hence they are more sensitive to 2DG treatment than non-cancerous cells.

2DG was one of the first proposed CRm, resulting in reduced circulating insulin levels and lowered body temperature, two physiological markers strongly predicting longevity, but producing at the same time detrimental cardiotoxicity in rats (Ingram & Roth, 2011; Minor et al., 2010; D. L. Smith, Jr. et al., 2010).

In addition to its glycolytic-inhibitory capacity, 2DG was found to induce endoplasmic reticulum stress (ER stress) through competition with mannose in the initial steps of N-linked protein glycosylation. Protein glycosylation begins in ER and is considered as a post-translational modification. The "N" in the name of this type of glycosylation indicate that the glycans are covalently attached to Asparagine (Asn or N) I am not sure about this, I thought that N is due to linking to nitrogen instead of oxigen, since there are also O-glycoproteins. It is the most common type of protein glycosylation - 90% of glycoproteins are N-glycosylated. Changes in surface protein glycosylation have been observed in the development of cancer and could serve as immunostimulatory signal (Stowell, Ju, & Cummings, 2015; Wolfert & Boons, 2013). In fact, it has been observed that 2DG treatment can affect T-cell and NK cell-tumor cell recognition and

activation *in vitro*, having opposite effects probably dependent on the dose of 2DG administered, enhancing cancer recognition upon low dose (0.5 mM) and decreasing it when the dose was relatively high (20 mM) (Andresen et al., 2012; Beneteau et al., 2012). In the second case, the inhibition of cancer recognition by NK cells was attributed to abrogation of NKG2D ligands expression on the cell surface, the mechanism that will be described further in the discussion.

- **Metformin**

Metformin, blood glucose lowering drug from the family of biguanides has been for long time the first-line medication for type 2-diabetes mellitus patients. Its primary mechanism of action is the ability to reduce hepatic gluconeogenesis and increase glucose turnover in muscles and adipose tissue in hyper-glycemic patients, which is primarily driven by AMPK activation (Pernicova & Korbonits, 2014). Mechanistically, metformin inhibits the mitochondrial respiratory complex I, leading to activation of AMPK, followed by enhanced insulin sensitivity and lowered cAMP levels. Metformin also has AMPK-independent effects on the liver that may include inhibition of fructose-1,6-bisphosphatase by AMP (Martin-Montalvo et al., 2013; Rena, Hardie, & Pearson, 2017; D. L. Smith, Jr. et al., 2010). In addition, in some context it can efficiently inhibit mTORC1 signaling independently of its action on AMPK, by which some of its physiological effects can be explained (Ben Sahra et al., 2011).

Metformin caught attention as cancer modulating treatment, due to emerging reports of lower incidents of tumor formation in diabetic patients receiving metformin, as well as lower mortality rates among those who already developed cancer (Landman et al., 2010). Even more interestingly, this effect turned out to be immune system dependent, since metformin treatment in immunodeficient mice did not delay either cancer growth or survival (Eikawa et al., 2015).

- **Rapamycin**

As already mentioned, rapamycin is the inhibitor of mTORC1, central hub of nutrient sensing in the cell. Its administration profoundly mimic the CR phenotype, including whole organism metabolism, gene expression and serum bio-profiling, leading to increased longevity in various animal models (Ingram & Roth, 2015). Unfortunately, the negative effect of rapamycin on

mTORC2 and side negative metabolic outcome render this CR mimetic as of very limited use (Fang et al., 2013; Lamming et al., 2012). Interesting observation is the upregulation of autophagy due to mTOR inhibition by rapamycin, which could promote removal of damaged and misfolded proteins (Fig 4).

- **Aspirin**

Another recently proposed CRm is the well known drug acetylsalicylic acid (Aspirin). It belongs to the non-steroid anti-inflammatory (NSAID) group reducing fever, pain and inflammatory response. For the discovery of the mechanism by which aspirin reduces inflammation, a Noble prize was awarded in 1982. Primary mechanism of aspirin is the suppression in the production of prostaglandins and thromboxanes by inhibition of cyclooxygenases COX-1 and COX-2, required for prostaglandin and thromboxane synthesis. Additional mechanisms of action include uncoupling of oxidative phosphorylation (Norman, Howell, Millar, Whelan, & Day, 2004), NO free radicals formation (Paul-Clark, Van Cao, Moradi-Bidhendi, Cooper, & Gilroy, 2004) and NF-κB signaling modulation (McCarty & Block, 2006).

Another important effect of aspirin molecular mechanism is the prevention of blood clot formation and blood thinning. By interfering with thromboxane production, it is reduces the platelets ability to form aggregates, thus hampering wound formation and healing.

It has been shown that low-dose aspirin treatment can mimic CR by stimulating autophagy through the inhibition of acetyltransferase EP300 by direct competition with acetyl coenzyme A to bind EP300 enzyme catalytic domain (Pietrocola, Castoldi, Maiuri, & Kroemer, 2018; Pietrocola, Castoldi, Markaki, et al., 2018). Unfortunately these promising results have to be taken with caution, as long-term aspirin administration risk can overshadow the potential benefits, and chronic administration of aspirin has been unexpectedly found to increase the cancer-related cell death risk (McNeil et al., 2018).

II. CANCER

1. Cancer – common denominator of many diseases

Cancer is not a single disease, but a group of malignancies caused by uncontrolled proliferation of heterogenous cell mass, sharing some common features which are known as hallmarks of cancer (Hanahan & Weinberg, 2000, 2011). The hallmark list initially included six features: proliferative signaling; growth suppression evasion; metastasis; replicative immortality; angiogenesis and cell death evasion; which was later expanded by additional four: immune escape, inflammation, metabolism deregulation and genome instability. Thus, obtaining the above-mentioned malicious character, the initially healthy cell must undergo a series of steps, reshaping its inner biological machinery, simultaneously evading recognition to be killed (by immune system in higher organisms) or to commit a suicide (by its own self-destruction machinery initiated by the defects caused by malignant transformation and stress).

It is not an easy path, that is why majority of pre-cancerous cells are detected and efficiently removed from the organism by its own surveillance system (Afshar-Sterle et al., 2014; Marcus et al., 2014). For the cell to succeed and evade all of the obstacles (internal and external) it can take years for the disease to manifest and be detected by the current methods (Valastyan & Weinberg, 2011). The time before cancer initiation and detection is called latency period. This latency period, estimated to be years (even decades) is the time where it is believed natural selection in the microscale is taking place for precancerous cells, followed by exponential growth and deadly metastatic processes when the tumor is finally detectable and reaches certain malignancy stage (Greaves & Maley, 2012). The dormant, slowly dividing pre-cancerous cells that do not yet have the potential and resources to overcome natural barriers and defences of the organism are much more prone to be targeted and removed at this early stage than later in time, when they are so much more heterogeneous and resistant. That is one of the rational for early cancer detection methods as a very important step in cancer elimination and treatment. Yet, as it was mentioned, years can pass without any visible symptoms of the emerging disease, and likely that is the exact time that all the prevention measures have the highest impact on stopping cancer progression. Hence, here we will

focus on couple critical steps in cancer developmental evolution – escaping cell death, escaping anti-cancer immune recognition, and reshaping cell metabolism to support growth and expansion.

2. Cancer - Avoiding death

In higher evolved organisms, removal of particular cells during certain developmental stage is natural and necessary, thus this process is precisely regulated on many steps. In cancer development, a premalignant cell has to deregulate death pathways to avoid cell suicide as a result of gene and protein damage accumulation. Therefore, tumor evolves two main mechanisms to progress: avoiding cell death in first place; and if that is not possible, uses apoptotic machinery to die in an immunological silent way, so the host organism will not be alarmed and respond towards malignant cells. Herein, the importance and character of apoptosis will be shortly described in the context of cancer and immune response, and the recent concept “immunogenic cell death” will be presented.

A. Apoptosis – programmed cell death

Apoptotic process is necessary for proper development of multicellular organisms, and as it has to take place in physiological conditions, without disrupting the normal growth and function of the tissue, death of the cell happens in a “silent” mode – no unnecessary damages to the bystanders. This type of death, a physiological self-destruction, was named apoptosis, and is characterised by a multi-step programmed process leading to decomposition of the cell structures and re-absorption by the neighbouring cells and professional phagocytic cells, without rendering stress and leaving trace of its existence, as opposed to cell necrosis, which was initially defined as a passive or accidental cell membrane disruption, leading to inner cytosol leakage and inflammation. In recent years, multiple cell death types have been discovered and this list is likely to be expanded and characterised more deeply in the near future (Galluzzi et al., 2018). From the cancer perspective, avoiding cell death is one of the first barriers to overcome.

Various stresses (extrinsic and intrinsic) are known to trigger the apoptotic program. Even the sole process of tumorigenesis can be internally exhausting and challenging enough to activate proteins and pathways governing self-destruction cell mode: elevated oncogene signaling, DNA damage

and acute cellular energetic deficiencies, for instance. Yet, those tumors that succeed in progression and resistance to the therapy managed to efficiently block activation of pro-apoptotic pathways (Fulda, 2010).

The apoptotic machinery can be triggered by sensing an intracellular signal (intrinsic program) or by receiving extracellular signal "to die" involving for instance, Fas receptor activation (extrinsic apoptotic program). Both programs culminate in activation of caspases, caspase 8 for extrinsic and caspase 9 for intrinsic program, which in turn activates downstream effector caspases family-3,-6, and -7 (**Figure 6**). The apoptotic process is characterized by:

- mitochondrial outer membrane permeabilization and cytochrome C release
- phosphatidylserine relocalization from inner to outer side of the cell membrane
- DNA fragmentation
- cell shrinkage and fragmentation
- ATP-dependence
- immunologically silencing – no inflammation

The intrinsic apoptotic program is more widely implicated as a barrier to tumorigenesis, but there is an increased interest of extrinsic cell death ligands use as a manner to induce cancer cell death in pre-clinical treatments.

The extrinsic apoptotic pathway begins when membrane receptors containing Death Domain (DD) such as TNFα receptor 1, FAS receptor or TRAIL receptor binds to its specific ligand, here TNFα, FAS-ligand or TRAIL, respectively. Receptor-ligand binding triggers the recruitment of proteins to the DD region in the cytosolic part of the receptor, forming the death-inducing-signaling-complex (DISC), initiating the apoptotic machinery, resulting in pro-caspase 8 activation via dimerization in so called induced proximity mechanism. Main function of activated caspase 8 is initiate cell death (type II extrinsic pathway). Thus, caspase 8 can cross-react with intrinsic

apoptotic pathway by favouring Bid cleavage and translocation to mitochondria, where it activates the intrinsic apoptotic pathway (Green, Galluzzi, & Kroemer, 2014).

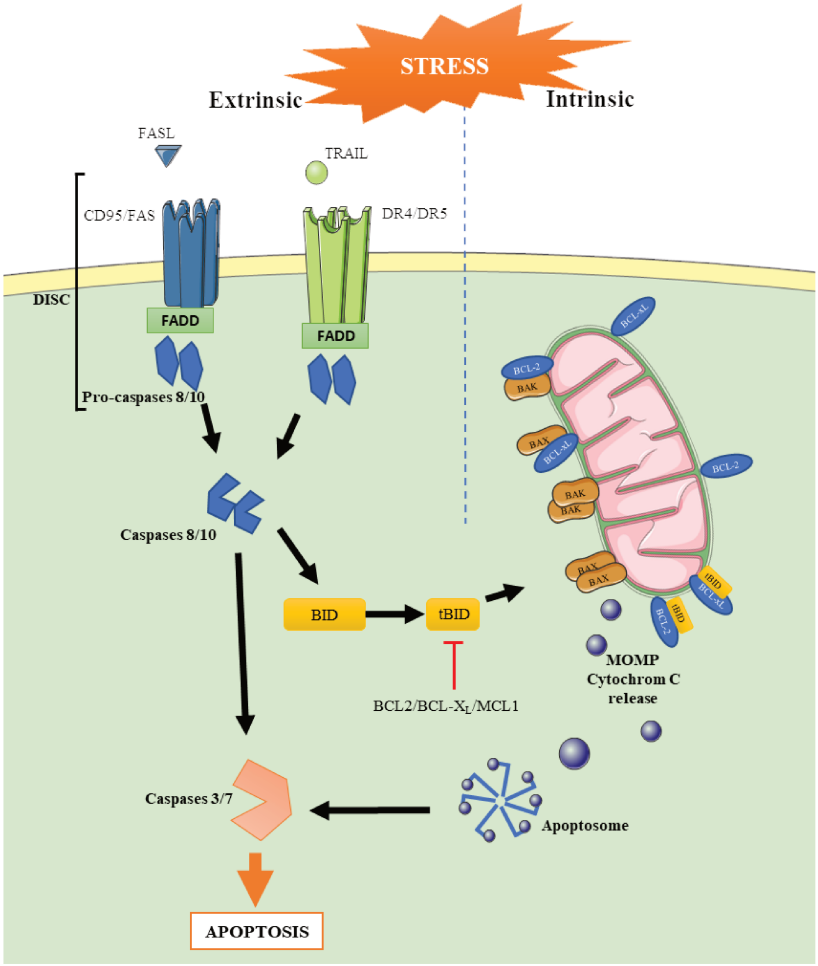


Figure 6: Intrinsic and extrinsic pathways leading to apoptotic cell death.

Apoptosis proceeds via two main pathways: death receptor-mediated (extrinsic) and mitochondria-dependent (intrinsic). The intrinsic pathway is triggered by specific receptor/ligand binding such as Fas and FasL or Tumor necrosis factor- related apoptosis inducing ligand (TRAIL) receptors with DR4 or DR5, followed by recruitment of adaptor protein FADD and activation of procaspase 8 and 10. The latter induces apoptosis by activating caspase 3/7 or by cleaving BID, bridging intrinsic pathway, resulting in mitochondrial outer membrane permeabilization (MOMP) and release of cytochrome C following by activation of executor caspases. The intrinsic pathway is regulated by levels of anti- and pro-apoptotic BCL family member, such as pro-apoptotic BAX and BAK and anti-apoptotic BCL-2, BCL-X_L and MCL-1. BCL-2—associated X protein (BAX), BCL-2 homologous antagonist/killer (BAK), and BID promote MOMP antagonistically to BCL2 family member action. Cytochrome C bind to Apoptotic Protease Activating Factor (APAF-1) to form a multimeric complex apoptosome that recruits and activates pro-caspase 9, an executioner protease that in turn activates caspase 3, resulting in cell apoptosis.

The intrinsic apoptotic pathway involves mitochondrial protein machinery, and it is responsible for majority of physiological cell death in higher organisms. In a non-physiological context (pathological), intrinsic apoptosis pathway triggers are: DNA damage, heat shock, UV, irradiation, oxidative stress, chemotherapy and ER stress among others. Briefly, the cell stress(-ors) can impact on the family of proteins governing the apoptotic machinery localized in the mitochondria, where the signal is integrated. The ratio between the anti- (i.e. Bcl-2 family, Bcl-xL, Mcl-1) and pro-apoptotic proteins (i.e. Bad, Bax, Bok, Bid, Bim) dictates the cell fate by regulating the integrity of mitochondrial outer membrane (MOM). When pro-apoptotic proteins prevail, MOM permeabilization (MOMP) leads to release of apoptosis proteins and cell death. Cytochrome c, an essential component of oxidative respiration that transfers electrons between Complexes III and IV in the inner membrane and is found exclusively within mitochondria under physiological condition, upon MOMP binds to APAF-1 and caspase-9 forming the apoptosome, activating caspase-9 and then executor caspases -3, -6 and -7 (Cotter, 2009). Apoptosome formation can be hampered by overexpression of proteins belonging to heat shock protein (HSP) family, mechanism which is widely utilized in cancer cells due to their higher than normal exposure to stress, and it is one of the cell death escape mechanisms acquired by some cancers (Beere et al., 2000; Bruey et al., 2000).

Following apoptotic cell self-disintegration, cells secrete and expose a variety of molecules that serve as the “eat me” signals, allowing to be recognized as well as to be eliminated, and “find me” signal, attracting the specialised phagocytic cells. These primary signals are present during the immunologically silent apoptotic cell death as they are in the necrotic death, and it is the co-existing secondary signals (cytokine and DAMPs) that eventually determine the immune response.

One of the major regulator shaping the character of the cell death, either pro- or anti-immunogenic, is the endoplasmic reticulum (ER) and pathway implicated in its stress response, further described in the following chapters.

B. ICD inducing chemotherapies

Immunogenic cell death (ICD) is a form of cell death which can induce an effective anti-tumor immune response (Krysko et al., 2012). Majority of ICD inducers, elicit danger signaling through ER stress/UPR signaling caused by some cytostatic agents such as anthracyclines doxorubicin (Doxo) and mitoxantrone (MTX), oxaliplatin, bortezomib, or physical stressors including radiotherapy and photodynamic therapy (Galluzzi, Buque, Kepp, Zitvogel, & Kroemer, 2017a; Garg & Agostinis, 2014; Krysko et al., 2012). The majority of commonly used chemotherapeutics fails to induce ICD. Chemotherapies that can induce canonical ICD through calreticulin (CRT) exposure turned out to have an immunogenic effect that is dependent on the ER stress-mediated induction of the PERK/eIF2α pathway which regulates the surface exposure of CRT (Obeid et al., 2007; Panaretakis et al., 2009).

Anthracyclines have been long used in the treatment of leukemia, lymphoma, uterine, ovarian, sarcoma, and breast malignancies. The most commonly used anthracycline is doxorubicin due to its low toxicity and potent antitumor activity that depends on $CD8^+$ T cells and IFN-γ, which in turn correlate with the response to the treatment (Mattarollo et al., 2011). In fact, the response of cancer cells to anthracyclines has found to partially mimic those induced by viral infection, as both induces an Type I IFN (IFN-I) response through Toll like receptor 3 (TLR3) activation. IFN-I antagonist is IFNAR1, which upon binding will trigger the release of CXCL10. In this sense, it has been shown that anthracycline efficacy can be reduced by neutralizing the IFN-I receptors (IFNAR1) and the CXCL10 receptors (CXCR3) (Sistigu et al., 2014). This could partially explain the enhanced response to chemotherapy of tumors growing in syngeneic immunocompetent mice rather than in immunodeficient mice.

Another example of an anticancer therapy that has been shown to have immunogenic activity is bortezomib (market name: Velcade). This proteasome inhibitor triggers damaged protein accumulation, inducing chronic ER stress through the activation of the PERK/CHOP pathway, which may sensitize cells to BOK-dependent apoptosis (Carpio et al., 2015; Llambi et al., 2016). It renders tumor cells immunogenic by upregulating HSP60 and HSP90 on the cell surface, improving dendritic cell (DC) function and inducing an CD8 T cell-mediated immune response (Chang et al., 2012; Spisek et al., 2007).

Interestingly, new experimental drugs with the capacity to induce ICD are being continuously discovered, like Epothilone B. This microtubular inhibitor cause polyploidy, induces ICD and results in anticancer immunosurveillance. It is being investigated for the treatment of ovarian cancer, lung cancer, brain cancer, breast cancer, and gastric cancer (Senovilla et al., 2012). Additionally, drugs with therapeutic uses other than cancer treatment are also starting to be discovered as ICD inducers with potential anticancer properties. This is the case of Digoxin and Digitoxin, which are cardiac glycosides used to treat heart failure. These cardiac glycosides induce the accumulation of cellular Ca^{2+}, which is beneficial to cardiocytes but ultimately leads to cytotoxic ER stress in cancer cells (Menger et al., 2012).

In the same way, chemotherapeutic treatments that do not induce danger-associated molecular patterns (DAMPs) exposure are unable to induce ICD. Cisplatin, for example, fails to induce ICD unless it is combined with ER stress inducers (i.e. thapsigargin, tunicamycin) (Martins et al., 2011) or with compounds that in combination will increase ER stress (i.e. pyridoxines) (Aranda et al., 2015). This was also the case when lymphomas were treated *in vivo* with a combination of Etoposide and 2-Deoxy-D-glucose (2DG). Etoposide alone did not induce an immune response unless combined with low doses of 2DG, which is a well-known inducer of ER stress (Beneteau et al., 2012).

Targeting the UPR in cancer treatment is an interesting and growing area of research. Nevertheless, these approaches may also alter the development and function of tumor infiltrating immune cells, affecting immunosurveillance and favoring immune escape mechanisms, for example increasing pro-tumoral cytokine-driven inflammation. For these reasons the combination of UPR-targeting drugs with chemotherapies should be carefully evaluated. Recent studies have demonstrated that induction of immune response along with non-immunogenic therapies can result in additional benefit and improved treatment response. This is the case of CR that was shown to induce the autophagic stress response when combined with non-immunogenic chemotherapies, resulting in an anticancer immune response (Pietrocola et al., 2016).

3. Metabolism of cancer cells

While the observation that cancer metabolism is abnormal in comparison to the healthy tissue was done almost a century ago by the famous Noble Prize winner Dr Otto Warburg, the metabolic modulation as an effective anti-cancer therapy has just started to deliver promising results. Dr Warburg noticed markedly increased glucose consumption by tumors, the cancer feature that is used routinely nowadays in cancer diagnosis by positron emission tomography (PET) imaging with radioactive labelled glucose analogues.

Just a quick glimpse at the simplified metabolism signaling pathway map could answer the question why so many people failed to harness its therapeutic potential – the complexity and sophistication of multiple intertwined circuits within a cell make it look particularly difficult to target. Metabolic treatment-induced modulation should be lethal towards malignant cells while sparing non-malignant cells. At a time, the genetic approach seemed much more attainable, and was holding a great promise to stop cancer at the very beginning. Unfortunately, cancer genetic landscape is much more complex than it was expected, and gene-based therapies are still in majority the promise of the future. Genomic landscape consists of deregulation and mutation of multiple genes, and probably much more of cross-gene interaction plus non-coding genome regions that are known/or very likely are affecting all the paths to tumorigenesis, not mentioning the constant micro-evolution happening within the heterogenous tumor tissue. On the other hand, cell metabolism analysis reveals limited number of ways that the cell can use to produce energy and building blocks.

The major two ways of ATP production by cells is glycolysis and oxidative phosphorylation (OxPhos) (**Figure 7**). It was often considered that proliferative cells rely mostly on glycolysis and differentiated cells use oxidative phosphorylation as the main source of energy.

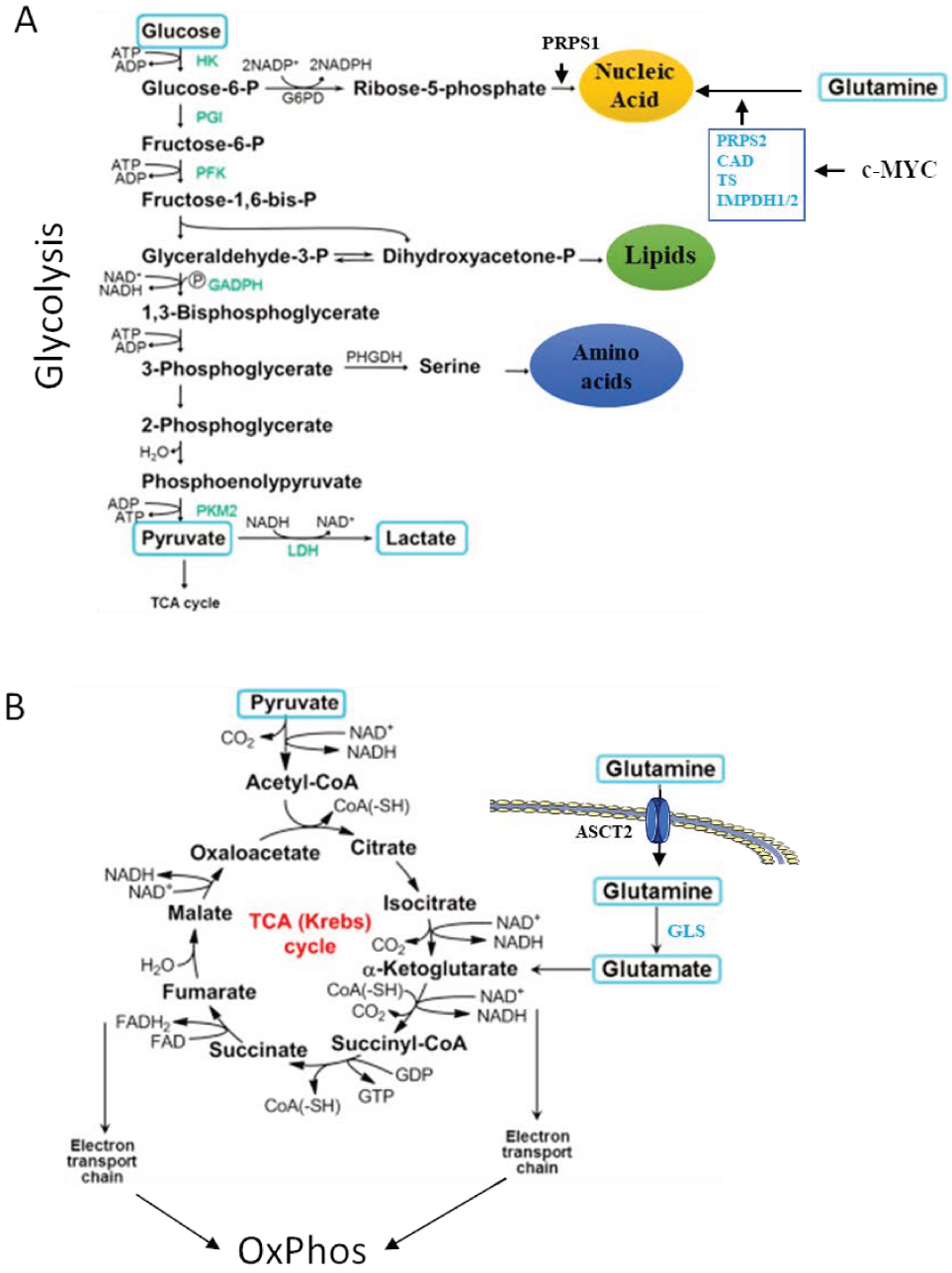


Figure 7: Central carbon matabolism pathways

Glycolysis (A) and Citric acid cycle (B). Adapted from (Kalyanaraman, 2017) Description in text. HK, hexokinase; PGI, phosphofructokinase; PFK, Phosphofructokinase-1; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; PKM2, Pyruvate kinase isozymes M2; LDH, Lactic Acid Dehydrogenase; PRPS1/2, phosphoribosyl pyrophosphate synthetase 1/2; CAD, carbamoyl-phosphate synthetase; TS, Thymidylate synthetase; IMPDH, Inosine-5′-monophosphate dehydrogenase; GLS, Glutaminase.

Glycolysis is a less efficient way of glucose utilisation in terms of ATP production- it gives merely 2 mol of ATP per 1 mol of glucose, whereas oxidative phosphorylation provides 36 mol/1 mol of glucose, but on the other hand glycolysis flux is significantly more rapid than OxPhos, partially compensating for its low ATP yield. Additionally, pyruvate, the end product of glycolysis, is subsequently converted into lactate using NADH as a source of proton, thus, replenishing the pool of free NAD^+ required for glycolysis to continue. Lactate is then removed extracellularly, not only being lost as a source of carbon but also creating a toxic environment. Surprisingly, Dr Warburg noticed that cancer cells are using glycolysis over oxidative phosphorylation even with unlimited access to oxygen (**Figure 8**) (Liberti & Locasale, 2016; Vander Heiden, Cantley, & Thompson, 2009). Although he proposed at that time that this could be the result of defects in mitochondrial functioning that would disrupt proper electron transport chain to function, nowadays we know that majority of tested malignant cells have perfectly functional mitochondria, implicating that glycolysis provides some advantage for rapidly proliferating cells over OxPhos. As it was later shown, healthy cells also preferentially use glycolysis under high proliferation demands, as for example rapidly expanding pool of T lymphocytes.

The explanation of this phenomena (preferential use of less energetically efficient glycolysis during proliferation) could be the much higher increase in demand of other nutrients (nucleotides, amino acids and lipids) for the proliferative cells, whereas the ATP demand changes just slightly. As it turns out, the energy might not be the limiting factor for highly dividing cells, but the building blocks and macromolecules as nucleotides and amino acids might be of much higher importance (**Figure 8**) (Vander Heiden et al., 2009). It is also the reason for high cancer glutamine dependency – as this amino acid is the direct precursor and anaplerotic supplier of the tricarboxylic acid cycle (TCA) for all of the above macromolecules. The important feature of cancer development is the fact that, at least in the initial phase, tumor cells are not limited by energy supply in form of glucose – as it is constantly delivered by circulation. In fact, the host ability to keep the constant levels of circulating blood sugars is enormous, even after prolonged food deprivation and starvation, ranging between 80 to 110 mg/dL, and rarely dropping under 70 mg/dL.

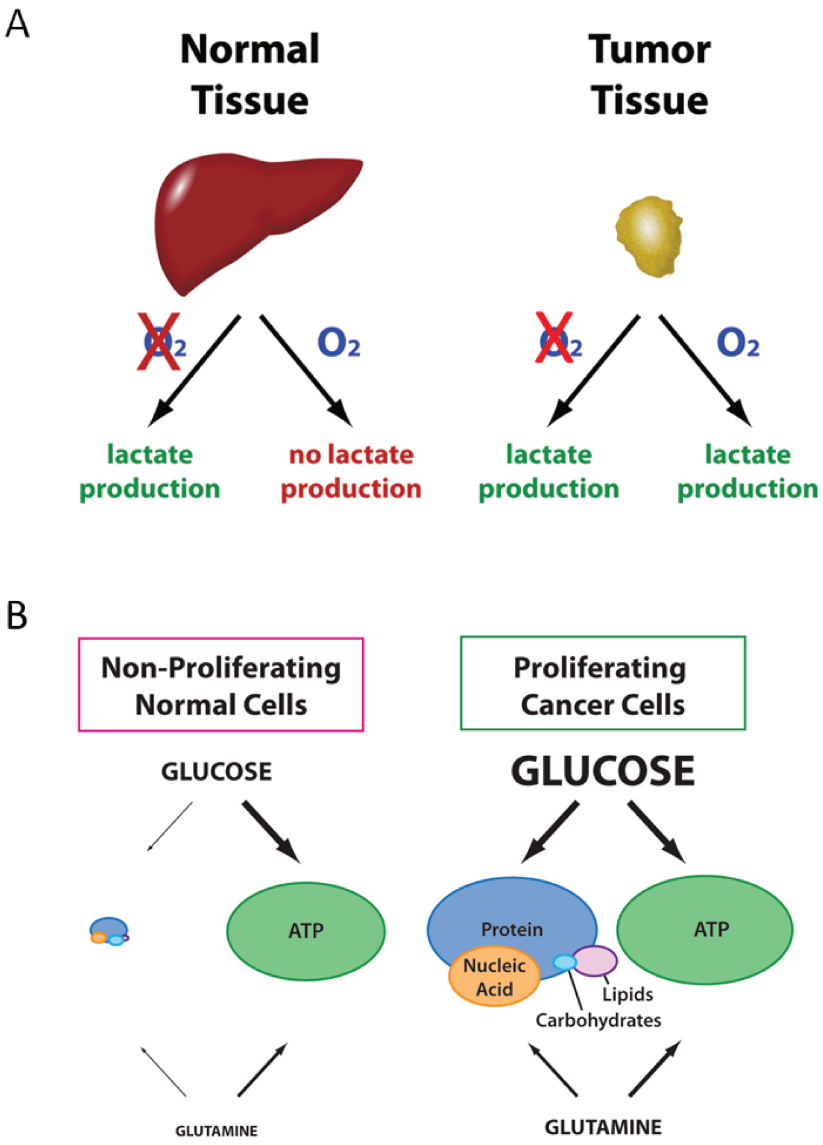


Figure 8: Warburg effect and proliferating cells nutritional requirements.

Tumor and proliferating cell metabolism as compared to normal/non-proliferating cell metabolism. (A) Normal tissue under hypoxic conditions switches their metabolism to glycolysis with the production of lactate as a byproduct, whereas when oxygen is available it will use it in oxidative phosphorylation and limiting lactate production. (B) Size of the boxes represents experimental utilization of specific nutrients and ATP in cultured cells. Most of the increased nutrient uptake in cancer and in proliferating cells is used to support biosynthesis. ATP use is relatively stable as compared between non-proliferating and proliferating cells. Adapted from Science Webinar series "Metabolic changes in cancer" 19.04.2012 Matt Vander Heiden, M.D., Ph.D.

Cancer cells exhibit an additional feature – their growth is independent from external signaling factors. As opposed to unicellular organisms, multicellular life evolved specialised interdependent tissues, whose particular development and growth is tightly regulated. Every tissue and organ has its own specialisation and function, making the organism as a whole unit more efficient, but also making every part dependent on the rest. Thus, cells growth and division is limited and occur only in response to extracellular growth signals, even though the cell is constantly supplied with nutrients, energy and oxygen. Hence, many cancer mutations lead to abnormalities in growth signaling, and pushes cell division in their absence, or even against the anti-proliferative signals.

A. Glucose

Cancer cells have a markedly increased consumption of glucose as compared to normal tissue. However, this overconsumption is used mainly in the *de novo* synthesis of lipids, nucleotides and amino-acids, instead of energy. Owing the necessity of malignant cells for glycolysis in order to produce sufficient amount of energy in parallel with an increased *de novo* synthesis, an important question arises: why and how tumor cells import so much extracellular glucose? Higher organisms evolved tight regulation of nutrient uptake via growth factor signals (Thompson, 2011). Deprived these signals, healthy cells do not survive *in vitro*, not able to import required nutrient even in an enriched nutritive environment (Aaronson, 1991; Talapatra & Thompson, 2001). Oncogenes that result in activation of PI3K/AKT signaling pathway enable to circumvent the need of external growth stimuli, thus enabling malignant cell survival (Aaronson, 1991; Edinger & Thompson, 2002). Such oncogenes act as master regulators of glucose uptake, inducing expression of glucose membrane shuttle GLUT1 and enhancing the activity of glycolytic enzymes hexokinase (HK) and Phosphofructokinase-1 (PFK) (Edinger, 2005; Pavlova & Thompson, 2016).

Based on this increased glucose consumption some therapeutic treatments like 2DG (discussed in chapter describing CR mimetics) have been tested, but unfortunately with low efficacy or with unfavourable side effects. Nevertheless, 2DG in combination with other chemotherapeutic drugs can result in additional synergistic benefits, for example sensitizing the tumor cells to the primary

therapy (Cheong et al., 2011; Meynet et al., 2012; Reyes, Wani, Ghoshal, Jacob, & Motiwala, 2017).

B. Glutamine – carbon source for lipid and nucleotide synthesis under hypoxia

Glutamine, a non-essential amino acid that is found at the highest concentration in the human bloodstream among other amino acids, similar to glucose it is tightly regulated and maintained at roughly 0.5 mM through the common effort of dietary uptake, *de novo* production and, if needed, muscle protein catabolism. In fact, rather than incorporating glutamine into protein synthesis, tumor cells use it as a source of nitrogen for *de novo* synthesis of nucleic acids and as a carbon source in anaplerotic fuelling of TCA cycle. Glutamine can be a precursor of multiple essential metabolites by their *de novo* synthesis (**Figure 9**).

Glutamine enhanced import and utilization is profoundly induced by transcription factor *c-Myc*. Its targets include glutamine transporters (ASCT2, SN2), glutamine to glutamate conversion enzyme (GLS1), as well as crucial enzymes in nucleotide biosynthesis - Ribose-phosphate diphosphokinase (PRPS2) and Carbamoyl phosphate synthetase II (CAD), thymidylate synthase (TS) and inosine monophosphate dehydrogenase 1/2 (IMPHD1/2) (J. Zhang, Pavlova, & Thompson, 2017) (**Figure 7**). All of the above-mentioned proteins promote the high metabolic flux and utilization of glutamine. In addition, the product of GLS1 reaction results in glutamate, a cell membrane impermeable metabolite, whose accumulation in turn serves as the exchange substrate for cysteine import by glutamate/cystine xCT/slc7A11 antiporter, supplying gluthatione synthesis and antioxidant response (Shin et al., 2017). On the other hand, invalidation of xCT protein improves cancer cell viability under glucose deprivation via preservation of intracellular glutamate to maintain mitochondrial respiration (Sayin et al., 2017; Shin et al., 2017).

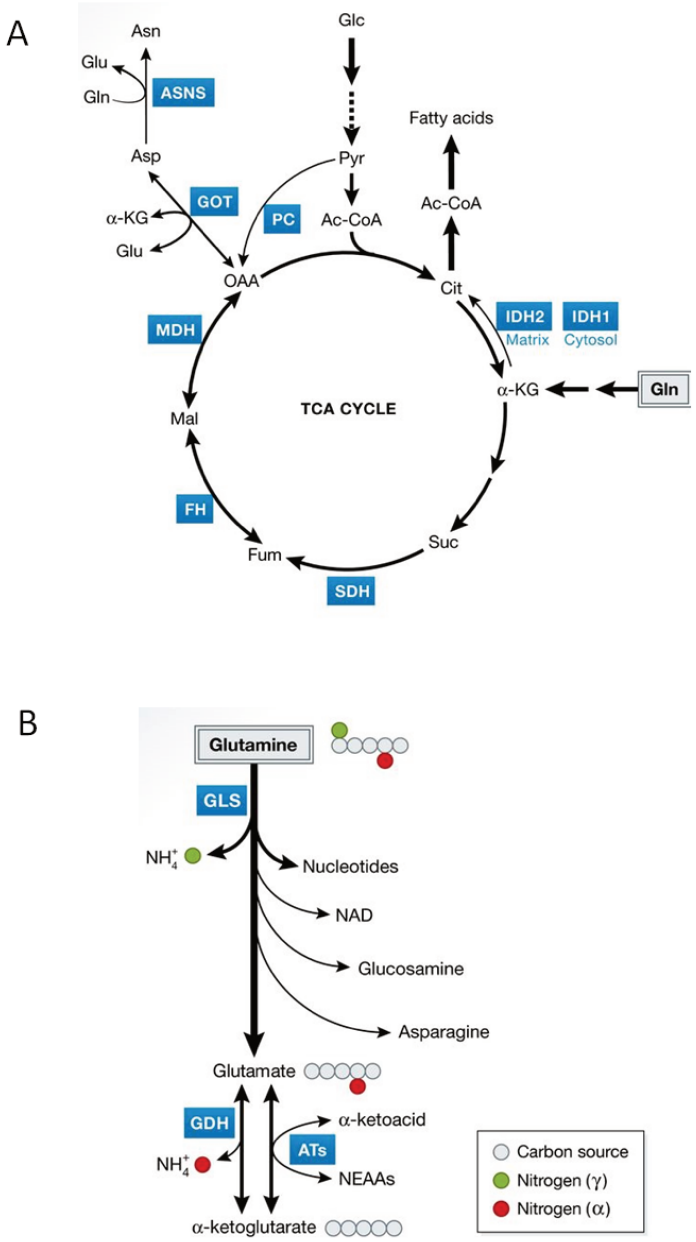


Figure 9: Glutamine as a precursor of various cellular metabolites.

(A) Entry of glutamine-derived carbon into the TCA cycle is via the conversion of glutamate into its α-ketoacid form, α-ketoglutarate. (B) Glutamine is an indispensable donor of reduced nitrogen for building both purine and pyrimidine bases. In addition, the γ-nitrogen of glutamine is also required to synthesize NAD, glucosamine-6-phosphate, and asparagine. Gln: glutamine; α-KG: α-ketoglutarate; Suc: succinate; Fum: fumarate; Mal: malate; OAA: oxaloacetate; Cit: citrate; Glu: glutamate; Asp: aspartate; Asn: asparagine; Glc: glucose; Pyr: pyruvate; Ac-CoA: acetyl-CoA; SDH: succinate dehydrogenase; FH: fumarase; MDH: malate dehydrogenase; GOT: aspartate aminotransferase; ASNS: asparagine synthetase; PC: pyruvate carboxylase; IDH1/2: isocitrate dehydrogenase 1/2. Adapted from Ji Zhang et al. EMBO J. 2017

In contrast to healthy cells generally importing exogenous fatty acids, many cancer types exhibit high rates of *de novo* lipid synthesis. Multiple oncogene-related pathways can impact on lipid biosynthesis (mTOR, AKT, IGF-1 to mention only few of them), partially by induction of lipogenic enzymes such as fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD1). It has been shown that tumor cells are highly depended on these rate limiting enzymes, and their inhibition can slow down progression of various tumors in mice (Ackerman & Simon, 2014; Currie, Schulze, Zechner, Walther, & Farese, 2013; Fritz et al., 2010).

Currently, a knowledge gap exists on tumor (geno-)types and their micro- and macro-nutrient requirements. There are numerous difficulties in studying metabolic alterations and their implications for tumor development. Additionally, cancer cells can have profoundly different metabolism *in vitro* versus *in vivo*. For example, KRAS-driven lung cancer is known to rely heavily of glutamine *in vitro*, but *in vivo* despite its presence, KRAS-driven malignant cells prefer to use mitochondrial metabolism of pyruvate entering the TCA cycle coming from glucose metabolism rather that through glutamine derived α-ketoglutarate (Davidson et al., 2016).

III. Immune system in cancer

1. Immune-selection and cancer progression

Cancer development can be divided into five stages: initiation, promotion, equilibrium (selection) and escape, followed by metastasis (**Figure 10**).

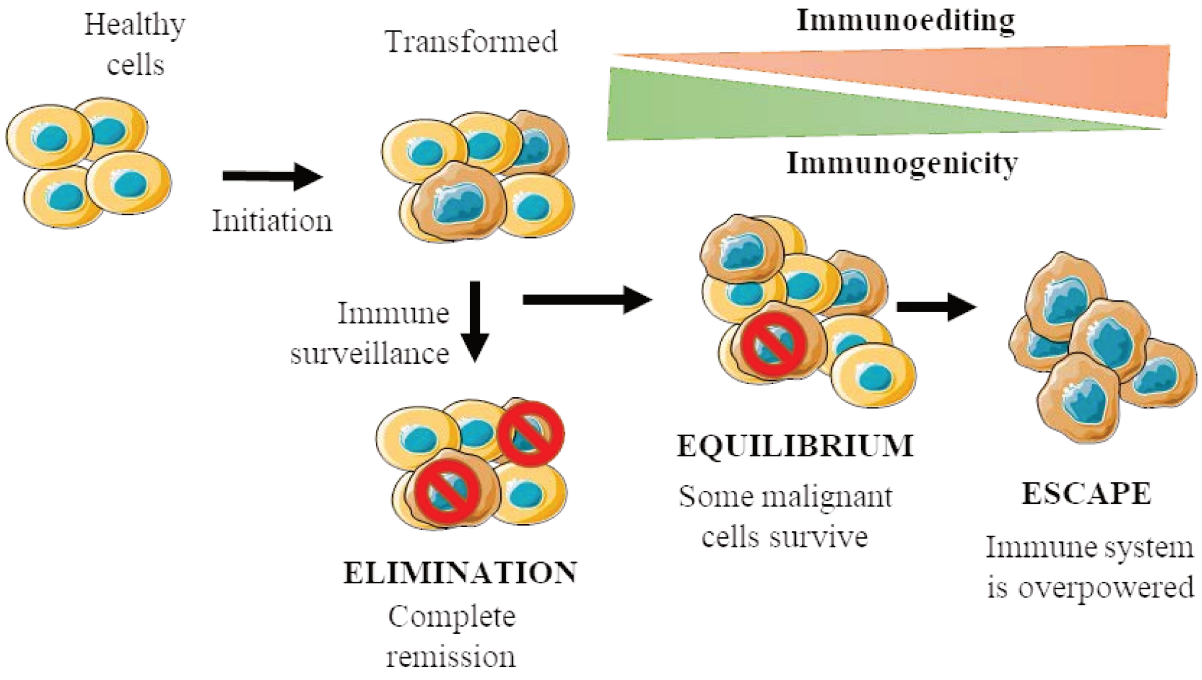


Figure 10: Cancer immunoediting stages

Stages of cancer development and their interdependence with immune response, leading to processes of immunoediting, which selects cancer cells and eventually tumors resistant to immune system response. At the early stages immunogenicity of malignant cells is high and most of them are eliminated. Microevolution favors cells with lower immunogenic phenotype to escape, hence selects for their disproportional to their immunogenic counterparts growth and dissemination. The last stage of this process is complete escape of low immunogenic cancer cells from the detection of immune system (Mittal, Gubin, Schreiber, & Smyth, 2014).

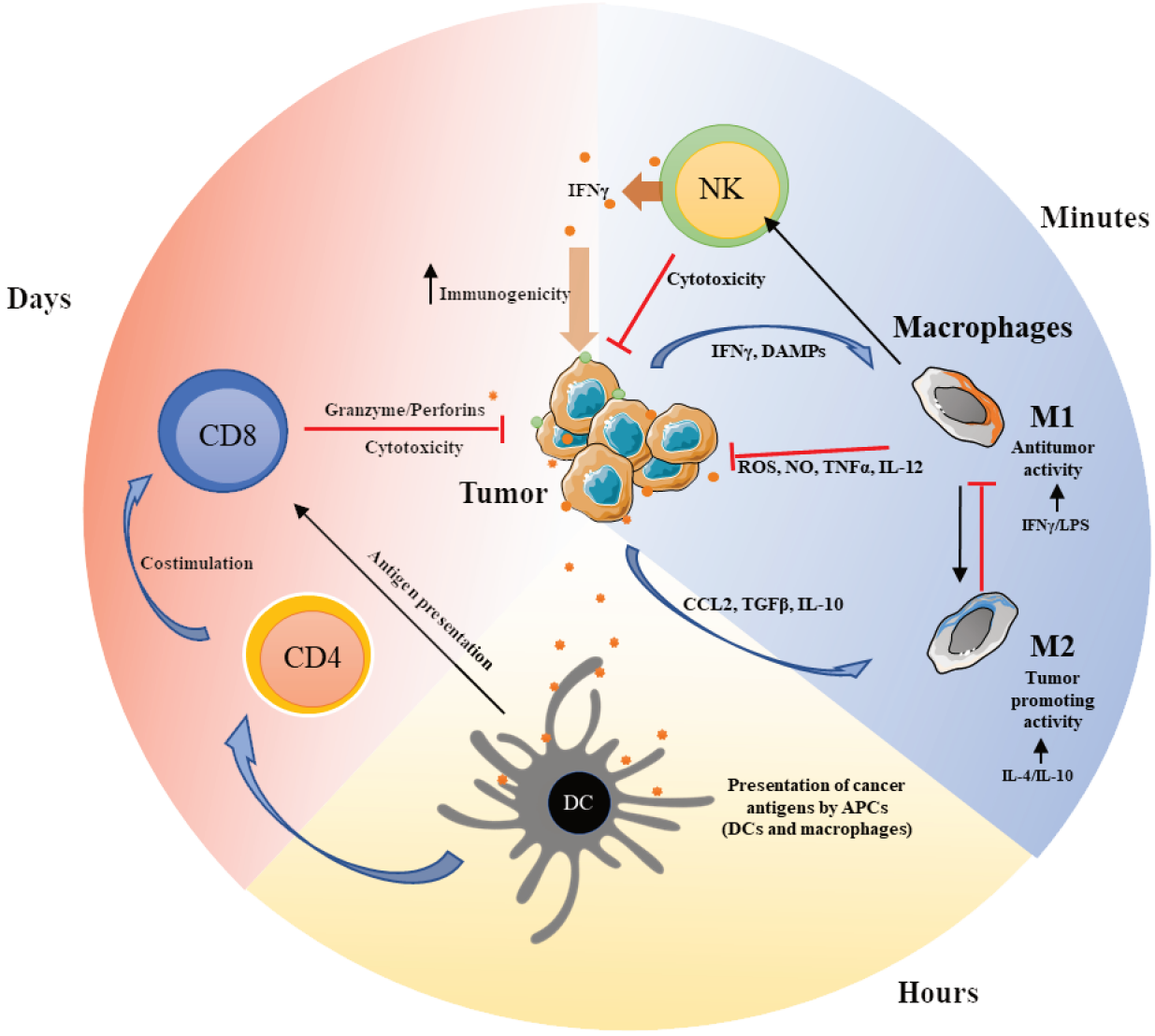


Figure 11: Innate and adaptive anti-cancer immune response cross-talk. Immune system in cancer.

Natural Killer (NK) cells and macrophages initiate quick anti-tumor response and shape TME in the following stages of cancer development. Importantly, APCs (DCs and macrophages) provide the essential link to trigger adaptive immune response by presenting antigens to effector $CD8^+$ T-cells with the help and helper $CD4^+$ T-cells. DC: Dendritic cells; DAMPs: Danger associated molecular patterns; ROS: reactive oxygen species; M1/M2: macrophages type I and II; IFNγ: interferon gamma; NO: nitric oxide; TNFα: Tumor necrosis factor alpha.

Initiation is the first pre-cancerous event of healthy cell transformation caused by oncogenic stressors, after which the majority of affected cells either undergo self-destruction or are recognised and eliminated by the innate immune system. Cells that escape from that pre-cancerous stage will

continue to acquire previously described cancer hallmarks, namely replicative immortality and abnormal metabolism, and will transit in the stage of cancer promotion. Here again, additional immune stimulatory triggers emerged from continued inflammatory processes will urge body defence mechanisms to fight, inducing local inflammation and increasing Tumor Infiltrating Lymphocytes (TILs), at which step many tumors can be rejected or suppressed and develop into benign tumors, no threatening life. Unfortunately, at this stage cancer cells also undergo negative immune-selection, eliminating highly immunogenic cells and favouring progression of the cells that developed low-immunogenic phenotypes. If the cancer cells find favourable circumstances and escape immune pressure, they will enter the last stages – escape and metastasis. The complete immune escape by tumor cells renders TILs entirely useless and exhausted, and the previous mechanisms of inflammation and cytokine secretion do not only lose their potential to suppress tumor growth, but they can even accelerate it.

Both innate and adaptive immune mechanisms play a role in tumor development, although T cells are found to be present within tumors three times more often (70%) than innate immune cells in human colorectal carcinoma (CRC) (Angelova et al., 2015).

Both innate and adaptive immunities play important role in anti-cancer response (**Figure 11**), and invalidation by genetic means either one of them cripple cancer cells elimination in a manner surprisingly reassembling pathogen elimination (**Figure 12**) (Klinke, 2012) as shown by O'Sullivan et al (O'Sullivan et al., 2012) in one of the pioneering studies investigating the theory of cancer immunoediting and dissecting the role of both arms of immune system in this mechanism. According to the immunoediting paradigm, the immune system recognizes and eliminates immunogenic malignant cells (capable of triggering an immune response), while omitting those that escaped that recognition. Cancer immunoediting is classically divided into 3 phases, elimination, equilibrium, and escape, starting with high immunogenic tumor sequentially progressing into last stage characterized by very low immunogenic cancer cells (**Figure 10**). Without immune system engagement, for example in the case of immunodeficient mouse models, immunoediting does not take place and cancer cells exhibit continuously highly immunogenic phenotype. Thus, immune system plays active and complex role along whole cancer development.

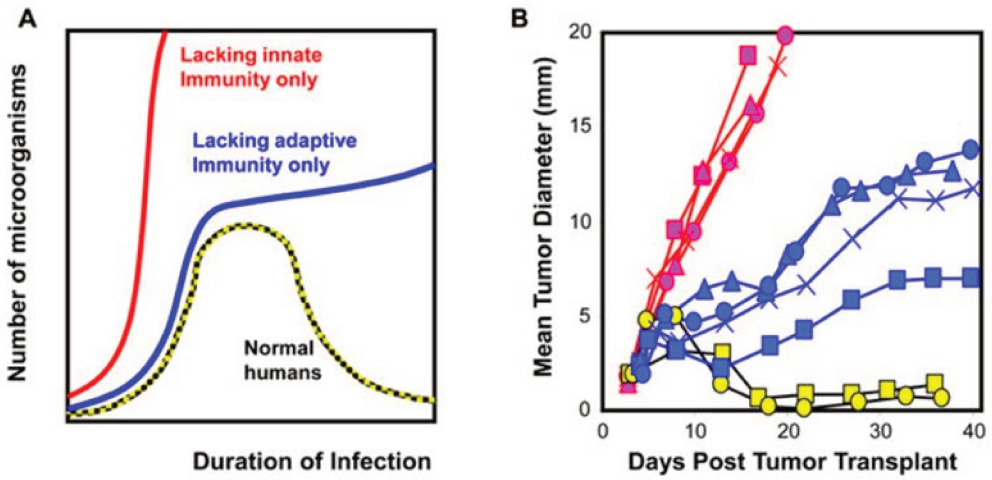


Figure 12: Innate and adaptive immunity responses.

Innate and adaptive immunity system responses against (A) pathogen infection and (B) cancer are of high resemblance. The yellow lines represent the normal full immune response against invassive or immunogenic cancer. In the absence of an innate immune response (red) the infected organism does not have time to develop adaptive responses and infection spreads quickly. Without adaptive branch, initially there is some control over infection initiated by the innate immunity, but later the infection perseveres and ultimately develops lethally (A). Similarly to (A), tumors from 3' methylcholantrene-induced sarcomas transplanted into syngeneic wild-type (yellow), $RAG2^{-/-}$ (blue), and $RAG2^{-/-}$x $\gamma c^{-/-}$ (red). Adopted from Klinke 2012.

2. Innate anti-cancer immune response

Innate immunity plays an important role in cancer development and immunomodulatory anti-cancer therapies. The innate immunity, evolutionary older than adaptive immunity, consists of anatomical barriers (chemical and physical), defence mechanisms (inflammation and complement activation) and specialised leukocytes: i.e. macrophages; dendritic cells (DCs); NK and γδ T cells (Mantovani & Sica, 2010; Peterson & Artis, 2014). In the recent years increasing amount of data connects innate immune cells involvement as important factor in triggering initial anti-tumor response and indispensable for subsequent activation of adaptive immunity. However, in many cases innate immune cells and inflammatory cytokines have been observed to have detrimental effects on tumor development, hindering T cell response and supplying tumor growth by constant

pro-inflammatory cytokine secretion (S. Lee & Margolin, 2011). In other words, it could be argued that the ineffectiveness of the transition from innate to adaptive immune response could result in tumor growth acceleration, as prolonged chronic inflammation, a hallmark of the innate immune system activity, in general promotes tumor growth and metastasis (Shalapour & Karin, 2015).

A. Dendritic cells

Dendritic cells (DCs) are specialised antigen-presenting cells (APCs) bridging innate and adaptive immunity. DCs are physiologically and functionally adapted to patrol crucial immunologically sensitive area of mammalian body - intestine, lung, skin (where they are called Langerhans cells). DCs are actively acquiring extracellular micro- and macromolecules, at the same time using the Toll-like receptors to detect any danger signals coming from pathogens or malignant cells (Nowarski, Gagliani, Huber, & Flavell, 2013). They are classically divided into conventional (cDC) and plasmacytoid (p) DCs. cDC are equipped with TLR2 and TLR4, sensing accordingly lipid-containing pathogen associated molecular patterns (PAMPs) (i.e. lipoteichoic acid and lipopolysaccharide, LPS) (Goubau, Deddouche, & Reis e Sousa, 2013); Y. Wang, Swiecki, McCartney, and Colonna (2011). Once activated, cDC secrete massive amount of IL12 which favours T cell growth, IFNγ and TNFα production and induces cytotoxicity of $CD8^+$ T lymphocytes and NK cells. IL10 within TME has been shown to counteract functionality and activity of DCs in the tumor context.

pDC are a smaller subclass of DC equipped with TLR7 and TLR9, recognizing single stranded RNA and pathogenic unmethylated CpG DNA, respectively. Upon activation, pDC produces type I interferons (IFN-I), IFNα and IFNβ (Goubau et al., 2013; Y. Wang et al., 2011).

B. Macrophages

Macrophages are highly specialised phagocytic cells actively scavenging and digesting cellular debris, foreign macromolecules, microbes, cancer cells, and any entities that do not express self-cellular surface markers. Macrophages are present in the TME at all stages of tumor development, and owing to their rich repertoire of mechanism and secretome they actively shape the TME and contribute to many aspects of cancer progression: inflammation, angiogenesis, metastasis,

immunosuppression, and in particular cases response to chemotherapy (Noy & Pollard, 2014). Experimental and clinical studies indicate that (at least in established tumors) macrophages generally favour tumor growth and act as immune system suppressors (Condeelis & Pollard, 2006; Kimura et al., 2007; Noy & Pollard, 2014). Hence, tumor-associated macrophage (TAM) depletion or reprogramming is of a high interest and an area of intense investigation. Two main macrophage phenotypes with antagonistic phenotypes have been described: M1 phenotype driven by IFNγ and LPS is pro-inflammatory and has anti-tumoral activity; and M2 phenotype driven by IL-4 and IL-10 with anti-inflammatory properties, associated with tumor progression and anti-tumor immune suppression (**Figure 11**) (Yuan et al., 2015).

TAM depletion with specific anti-CSF-1R antibody (binding and disrupting extracellular survival signal which leads to cell death) proved to effectively inhibit cancer progression in a mouse model of MC38 colorectal adenocarcinoma and MCA1 fibrosarcoma. Efficient depletion of TAM lead to increase in cytotoxic $CD8^+$ T lymphocytes and reduction in FoxP3 regulatory T cells (Tregs), resulting in decreased tumor growth and longer survival. Importantly, patients with metastatic primary pleural mesothelioma, endometrial carcinoma, and colorectal cancer receiving anti-CDF-1R therapy experienced partial response and exhibited $CD8^+$ T cell increase similar to experimental studies (Ries et al., 2014).

Important feature of this approach is the specificity of anti-CSF-1R treatment towards M2 macrophages (Cannarile et al., 2017). Furthermore, CSF-1R expression is detected on other myeloid cells within the TME, including DCs and MDSCs, however the effect of CSF-1R targeted therapy on these populations is still unclear (Cannarile et al., 2017).

Currently CSF-1R is under investigation in phase I/II for melanoma patients with a BRAF V600E or BRAF V600K mutation (NCT03101254).

Recently dietary protein restriction (21% protein in Ctr versus 7% in protein restricted diet) has been shown to slow down tumor progression in two independent syngeneic mouse models of prostate cancer RP-B6-Myc and kidney renal adenocarcinoma (RENCA) upon combination with anti-PD-1 therapy (Orillion et al., 2018). Protein-reduction diet did not alter the overall TAM infiltration, but increased the number of M1 macrophages in parallel with a decrease in M2

macrophages in human xenograft prostate cancer LuCaP23.1 SCID mouse model. These results are in accord with previous findings where low protein diet reduced tumor growth in these model, which was linked to decreased tumor proliferation and mTOR activity of cancer cells (Fontana et al., 2013). In addition, upon low protein diet combined with anti-PD-1 treatment, TME has increased ration of M1/M2 macrophages. Based on this promising result, a pilot clinical study was launched where patients receiving immunomodulatory treatment of prostate cancer vaccine for castration resistant form of prostate cancer will receive either 10% or 20% protein-containing diet during the treatment period, after which immune system will be evaluated by flow cytometry (Orillion et al., 2018).

3. Adaptive anti-cancer immune response

Unlike innate immunity, adaptive immunity is much more specific towards pathogens or stressors, but it needs time and diverse inter-cellular cooperation to properly develop. Importantly, it provides so called immunological memory, entrusted in long-lived resting T cells with the high potential to be re-activated upon antigen recognition and elicit an effective immune response. It is heavily based on recognition of short amino-acid segments (antigen) in the context of the major histocompatibility complex (MHC) class I through T-cell receptor (TCR). In the context of cancer, it has been shown that there are many of so-called neo-antigens, the products of defective protein synthesis caused by DNA mutations and alternative splicing. Specialized APCs are participating in effective education of T cells by active up-take of circulating antigens/proteins, degradation, processing and loading onto MHC class II complexes in order to present them to $CD4^+$ T cells which will elicit B-T cell cooperation for antibody production against the antigens. The alternative mechanism that is recognizable as of high importance in tumor immune response is the ability of APC to load acquired foreign antigens on the class I MHC (instead of MHC class II) complexes and cross-present them to $CD8^+$ T cells directly.

A. T lymphocytes

Bone marrow derived, thymus matured and selected T lymphocytes are present in the majority of solid tumors and are critical for an effective immune response and long-term tumor-free survival.

They express a range of surface receptors regulating their ability to engage, recognise and eliminate dysfunctional cells. Importantly, T cells can undergo a selection process in the peripheral lymph nodes, where with the cooperation of DCs and helper $CD4^+$ T cells they can gain potent and specific cytotoxic functions. But even then, inhibitory signals can overpower the cytotoxic signaling and render them ineffective in the tumor context, even though they retain most of their functional and proliferative abilities and cytotoxicity when tested *ex vivo* (Daud et al., 2016; Mahnke et al., 2012; Williams et al., 2017).

Cytotoxic T cells express T-cell receptors that after selection and maturation with the help of APC and $CD4^+$ T cells can recognize a specific antigen in the context of class I MHC molecules, normally present on the surface of all body cells. Upon positive recognition, T cell launches its killing machinery in the form of cytotoxic proteins perforins and granzymes which enters the targeted cell and initiate its programmed cell death. Another way to induce cell death is via surface interaction of FAS ligand and FAS receptor, which initiates the DISC recruitment and subsequent death machinery as described in chapter “Apoptosis – programmed cell death”.

In general, higher percentage of TILs is correlated and predicts a better clinical outcome, longer survival and higher chances of tumor-free survival (Naito et al., 1998; Pages et al., 2005; L. Zhang et al., 2003). However, other studies have demonstrated that some of the immune cell subsets are associated with worse prognosis, seeming to promote tumorigenesis. For example, Tregs can potently inhibit immune activity in TME, and paralyze CD8+ T lymphocytes cytotoxicity. Implication of $CD8^+$ in anti-cancer immune response will be further described in following chapters.

B. MDSCs and Tregs

Myeloid derived suppressor cells (MDSCs) can also hampered anti-cancer immune response through secretion of multiple immunoinhibitory signals, more specifically by secreting arginase I. Arginase I can extracellularly deplete arginine levels, making it less available for T cells, thus lowering their effectivity (Rodriguez et al., 2004).

Additionally, MDSCs have been shown to drive apoptosis of $CD8^+$ T cells, limiting their ability to immunosurveillance and respond to immunotherapy. Mechanistically, increased IFNγ levels within TME induced the pro-apoptotic FAS ligand expressed on MDSCs. Disruption of this FASL/FAS interaction let to enhanced immune response and synergistically with checkpoint blockade slowed down the growth of induced melanomas in mice (Horton & Gajewski, 2018; Horton, Williams, Cabanov, Spranger, & Gajewski, 2018).

Tregs, MDSCs and cancer cells by themselves supress effective immune response launched by TILs. TME promotes TILs apoptosis and poison immune effector cells through the release of factors like nitric oxide (NO), reactive oxygen species (ROS), IL-10, IL-6, arginase-I (ARG1), Vascular Endothelial Growth Factor (VEGF), indoleamine 2, 3-dioxygenase (IDO), and TGF-β (**Figure 13**) (Lippens et al., 2016; Monu & Frey, 2012; Zhai et al., 2018; Zhao et al., 2016). The cross-talk between immune cells population involving specific sub-population of DCs and Tregs is another layer of complexity, participating in the fine tuning and self-tolerance activation.

By secretion of IDO, pDCs promote Tregs immunosuppressive functions, ultimately halting immune response (Lippens et al., 2016). The current knowledge about development and regulation of cancer immunity, especially the immunosuppression by TME, is still incomplete and is likely to vastly expand in the coming years. Complexity and entanglement of various cells, cytokines and mechanisms involved in shaping immune response is probably one of the most challenging and promising area of investigation in order to improve and develop new strategies in cancer therapies.

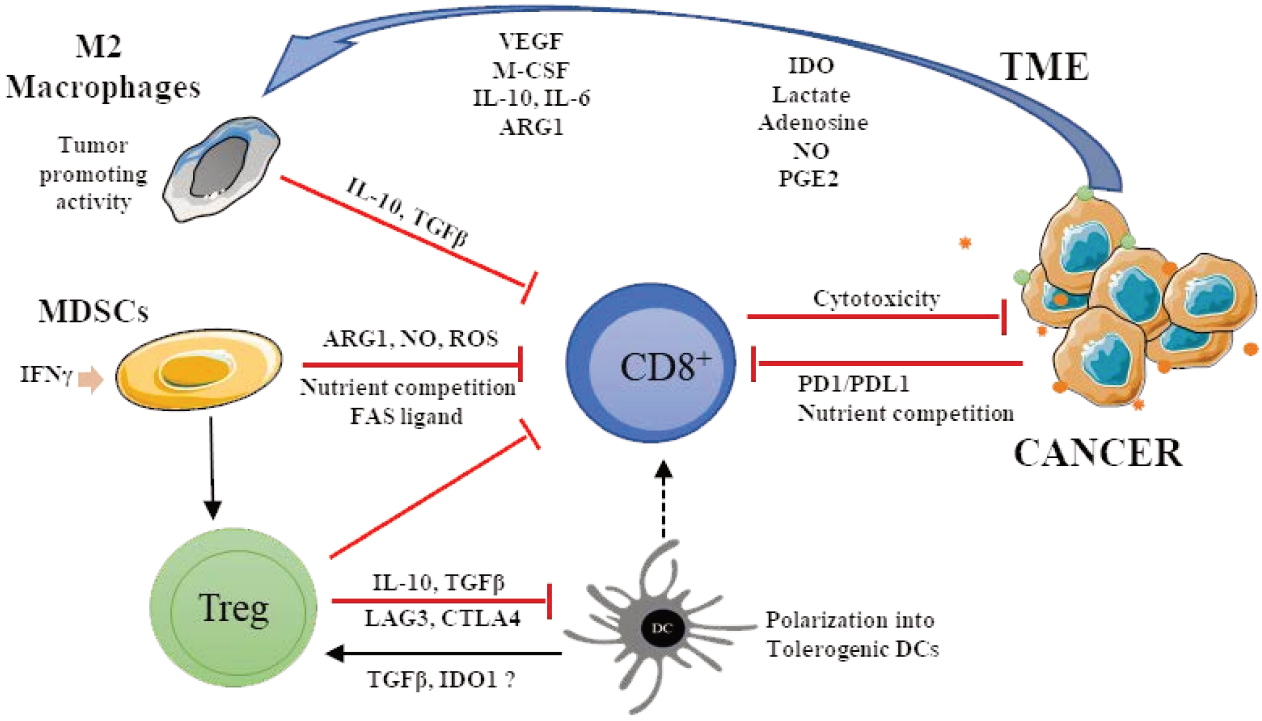


Figure 13:Immunoinhibitory signaling in cancer.

Mutiple signaling pathways contribute to deplete or inhibit effector immune cells. Cancer cells secrete chemokines, cytokines and oncometabolites to recruit and promote immunosuppressive cells such as myeloid-derived suppressor cell (MDSC), regulatory T cell (Treg) and tumor-associated macrophage type II (M2) to generate an immunosuppressive tumor microenvironment. These immunosuppressive cells directly suppress the cytotoxic functions of $CD8^+$ cytotoxic T lymphocytes through the expression and production of various factors and ligands. Tumors also actively inhibit functionality of $CD8^+$ cytotoxic cells by expression of surface immune inhibitory markers like PD-L1 and competing for nutrient which hamper T cell activity. MDSC: myeloid-derived suppressor cell; M2: tumor-associated macrophage type II; Treg: T regulatory lymphocyte; ARG1: Arginase 1; NO: nitric oxide; ROS: reactive oxygen species; IDO: Indoleamine 2,3-Dioxygenase 1. See further description in text. (Zhao, Wu, Shao, Shi, & Zhao, 2016)

4. Major ways of tumor immune escape

Since the start of the use of immunomodulatory therapies in cancer treatment (checkpoint blocking antibodies against PD-1 and CTLA4), two main groups of patients emerged - high and low responders. Quickly after it became clear that individuals with the major benefits exhibited

markedly different TME and TILs compared to patients lacking clinical response. The main characteristic of the high responder group is the high presence of $CD8^+$ T cells coupled with type I interferon (IFN-α/β) signature (Joyce & Fearon, 2015; Spranger, 2016). Based on that, cancer patients started to be sub-grouped into T-cell-inflamed and non-T-cell-inflamed patients **Table 2**.

Table 2: T-cell inflamed versus T cell-non inflamed tumor phenotype

TME Phenotype	T-cell-inflamed	non-T-cell-inflamed
T cell infiltration	high	low
PD-L1 / PD1 signaling	high	low
tumoral IDO expression	high	low
Tregs recruitment	high	low
surface antigen expression (i.e. MHCI)	low	high
CXCL9/CXCL10	high	low
PTEN status	mostly active	lost
innate immune recognition	present	non detectable
P53 signaling	present	lost
Beta- catenin signaling	low	high
CCL2/CCL4 expression	high	low
response to immunotherapy	good responders	bad responders

T-cell-inflamed tumors are characterized by the upregulation of immune inhibitory mechanisms, such as induction of immune inhibitory checkpoint on their surface, which makes them prone to immune checkpoint targeted therapies, in contrast to non-T-cell-inflamed tumors. Immune checkpoints are surface regulators of the immune system activation. Under normal circumstances, their action is crucial for developing self-tolerance. Some of them have been recognised as of high importance in anti-tumor immunity and are clinically targeted by specific monoclonal antibodies in order to re-activate anti-tumor response. CTLA-4 (expressed on Treg and activated T cells), PD-1 (expressed on T cells) and LAG3 (expressed on activated T cells, NK, B cells and pDCs) are the most common known inhibitory checkpoints. Various tumors upregulate ligands for immune checkpoint inhibitors, such as PD-L1 and CD80 in order to suppress anti-cancer immunity (Juneja et al., 2017; Lim et al., 2017).

Unfortunately, exclusion of anti-tumor specific TILs represents even more a therapeutically challenging mechanism of immune escape. Importantly, there is increasing amount of evidence that TME and stress sensing pathways can effectively modulate the transition between these two distinct tumor phenotypes. It is the area of ICD and secretory pathway modulation (chemokines and DAMPS) that will be extensively discussed in the following chapters. Nevertheless, these two major phenotypes of TME, $CD8^+$ T and $CD8^-$ T cells require significantly different approaches in order to maximize therapeutic effects.

5. Cytokines in cancer progression

William Coley, famously named “father of immunotherapy”, at the end of the XIX century made the striking observation that tumors can underwent spontaneous regression in patients infected by pathogen. By brilliant insightful idea, Coley attributed the cancer regression to the activation of the organism immunity, that was triggered by concomitant pathogen invader. Unfortunately, against intense efforts of its inventor, the clinical benefit of such discovery appeared way before scientific understanding and techniques could support the pursuit of effective and safe use of that observation.

Now we know that some of the response that Coley was observing was attributed to acute inflammation caused by infection, and as this may be initially successful in cancer therapy, very often tumors survive and even evolve the ability to feed on sustained inflammation as a way to promote their own growth. This is one of the reason by which pro-inflammatory agents has to be used with caution in patients suffering from cancer, but on the other hand, knowing why some cancer can be stopped by inflammation and why others thrive on it is critical to decide how to treat cancers effectively with immunotherapeutic-based approaches.

Interestingly, IFN-I production can be driven by retinoic acid-inducible gene I (RIG-I). An increase in IFN-I was found to favour anti-cancer T cell response resulting in spontaneous tumor rejection in some cases (Burnette et al., 2011; Diamond et al., 2011; Fuertes et al., 2011). In fact, both DCs and $CD8^+$ T cells have been found to rely on IFN-I to establish responses against tumor cells. In addition, it has been also demonstrated that IFN-β is an indispensable element of effective anti-

tumor response to immunomodulatory therapy *in vivo* by increasing DCs cross-presentation and drastically improving anti-PD-L1 treatment (X. Yang et al., 2014).

Inactivation of IFNα receptor in melanoma and colorectal cancer cells resulted in accelerated tumor growth in mice xenograft models and low expression of IFNAR predicted poorer prognosis in human CRC patients (Katlinskaya et al., 2016; Katlinski et al., 2017). This detrimental effect was mediated by generation of immune excluded (non-T-cell inflamed) tumors and attenuated anti-cancer immune response.

6. Nutrient competition within TME

Highly proliferating and hypovascularized solid tumors suffer from nutrient scarcity, creating hostile and competitive survival environment. Variations in extracellular nutrients can shape the metabolism and efficacy of immune cells occupying TME. In addition, the by-products of tumor metabolism can serve as signals for immune cells, modulating their response. High consumption of glutamine by enhanced glutaminolysis in tumor cells could result in its scarcity for TILs (Jin, Alesi, & Kang, 2016; Perez-Escuredo et al., 2016). Glutamine serves as an important factor in T cell function and self-renewal, and its extracellular level is indispensable for tumor suppressor metabolite S-2-hydroxyglutarate production in $CD8^+$ T cells (Sinclair et al., 2013; Swamy et al., 2016; Tyrakis et al., 2016).

TILs suppression can also be an effect of essential amino acid degradation by overexpression of indoleamine-2,3-dioxygenase (IDO), enzyme converting tryptophan to kynurenines (Rodriguez et al., 2004; Sinclair et al., 2013; Uyttenhove et al., 2003). More studies have investigated the critical role of various amino acid levels on T cell anti-tumor efficacy, but there is still a huge gap in our understanding of TME and its metabolic fluctuations. It is thought important to take amino acid impact on the immune cell population under consideration before proceeding with metabolic targeted therapies in cancer treatment. For example, serine deprivation slows down Myc-driven tumor growth, partially by making cancer cells less fitness to sustain environmental stressors, thus serine metabolism and bioavailability recently caught attention as promising therapeutic metabolic target (Maddocks et al., 2017; Sullivan & Vander Heiden, 2017). However, at the same time serine

seems to be particularly an essential metabolite for T cell effector expansion, hence likely affecting anti-tumor immune response (E. H. Ma et al., 2017).

Oxidized lipids, lipid metabolism and cholesterol levels are also important factors in TILs function. There is enormous amount of evidence that obesity, high fat diet, accumulation of excess fatty acids and its metabolism are strongly linked to cancer development. The surprising observation linking statin use and lower cancer incidents are gaining more attention (Demierre, Higgins, Gruber, Hawk, & Lippman, 2005; Gronich & Rennert, 2013). Lipid accumulation can impair DCs ability to effectively present and activate T cells (Cubillos-Ruiz et al., 2015; Ramakrishnan et al., 2014). In addition, to hampering T cell directly, oxidized lipids seem to fuel tumoral MDSC, further attenuating effective immune response (Al-Khami et al., 2017; Condamine et al., 2016).

IV. Endoplasmic reticulum, ER stress and UPR

1. Protein synthesis and ER stress

Protein synthesis is the most energetically demanding process in the cell, which consumes up to 75% of the overall cell energy expenditure (Lane & Martin, 2010). Yet, a significant amount of newly synthesized proteins does not fulfill the quality control standards and need to be eliminated or recycled. The damaged proteins, in addition to be energetically expensive, pose threats for cell physiology and homeostasis especially under the harsh environmental conditions of the tumor microenvironment (TME) (Hetz, Chevet, & Oakes, 2015).

Tumors are a non-homogenous mass of malignant and non-malignant cells residing within the TME, characterized by hypoxia, nutrient deprivation, lower pH and rich cell-to-cell interactions (Cubillos-Ruiz, Bettigole, & Glimcher, 2017). In order to progress and spread, the tumor need constant adaptation to changing conditions, which requires, among other things, enhanced protein production for various purposes. On the other hand, non-cancer cells are forced to modulate their metabolism either to adapt to current environmental circumstances, or to produce molecules intended to fight tumor expansion. In both cases, mechanisms altering cell proteostasis (proper protein homeostasis) are initiated to satisfy higher than physiological demand on protein production, and if these mechanisms are not sufficient, cells will experience endoplasmic reticulum stress (ER stress) as a result of the accumulation of misfolded protein within the endoplasmic reticulum (ER) (Ackerman & Simon, 2014; Cubillos-Ruiz et al., 2017).

The ER is a highly organized organelle with diverse functions, including lipid production, calcium homeostasis, and drug detoxification, but its main function is synthesis of roughly one third of all proteins (Hetz, Chevet, & Harding, 2013; Hur et al., 2012). For that reason, the ER is equipped with the biochemical machinery designated to promote proper protein maturation and folding, assess protein quality and direct defective proteins to repair or degradation pathways (Hetz et al., 2015). Protein modifications occurring in the ER are also one of the first steps of the secretory pathway, which includes protein glycosylation and trafficking of proteins expressed on the cell surface or delivered to the extracellular milieu (Dejeans et al., 2014; Galluzzi et al., 2017a).

2. Unfolded Protein Response

The unfolded protein response (UPR) is controlled by three ER stress sensors, all localized in the ER transmembrane: Activating Transcription Factor 6 (ATF6), Inositol Requiring Enzyme 1 (IRE1α) and PKR-like endoplasmic reticulum kinase (PERK) (Fig. 14). Under stress-free conditions, they are kept in their inactive form by the ER chaperone GRP78 (also known as BiP), which dissociates in the presence of misfolded protein accumulation in the ER, freeing the sensors and allowing the subsequent activation of the UPR signaling (Hetz et al., 2015). IRE1 is the first discovered and evolutionarily the most conserved UPR sensor, possessing both a serine/threonine kinase domain and an endoribonuclease domain. IRE1 is located in the ER membrane and when released from GRP78 repression, undergoes di/oligomeryzation and autophosphorylation, enabling its enzymatic activity. Subsequently, endoribonuclease activity of IRE1 catalyzes XBP1 mRNA alternative splicing, cutting out an intron of 26 nucleotides, which results in an open reading frame shift. The translational product of this alternatively spliced XBP1 (XBP1s) is a potent transcription factor, which targets genes encoding proteins participating in protein folding, in the ER-associated degradation and ubiquitin–proteasome pathways (ERAD), in protein trafficking, and in lipid biosynthesis (H. Kim, Bhattacharya, & Qi, 2015).

Additionally, the endoribonuclease domain of IRE1α has the activity to cleave and degrade various mRNA in a process named regulated IRE1-dependent decay (RIDD), thus reducing the overall synthesis load on the ER, but paradoxically it can also cleave genes encoding chaperone proteins, favoring apoptosis (Han et al., 2009).

Another branch of the UPR pathways is driven by PERK, a Ser/Thr kinase whose oligomeryzation state followed by autophosphorylation induces concomitant activation, reducing protein translation via phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α). Simultaneously, accumulation of peIF2α triggers the selective translation of ATF4, which is a transcription factor that can induce CHOP and GADD34, proteins engaged in apoptosis induction and peIF2α dephosphorylation,

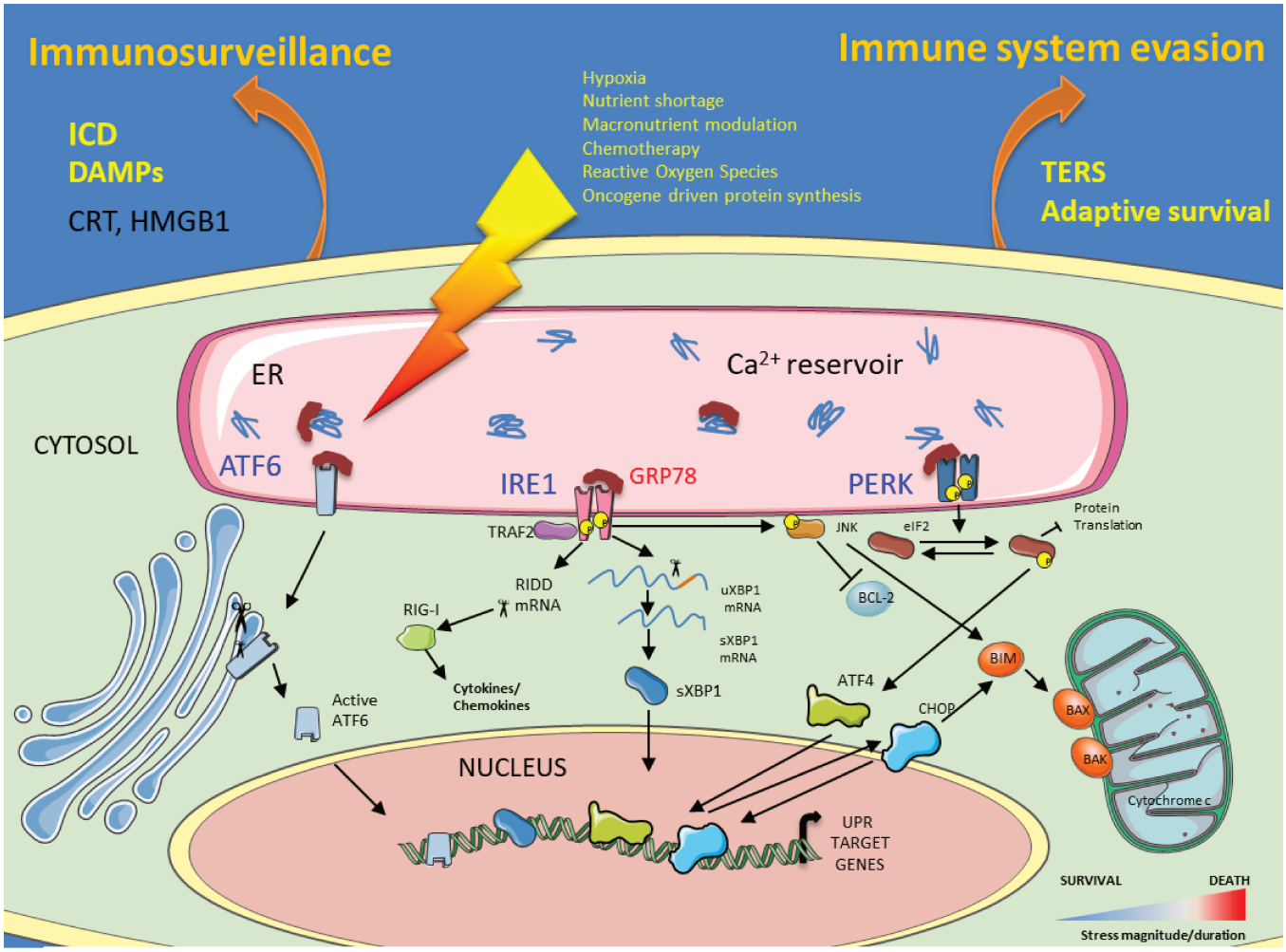


Figure 14:Unfolded Protein Response signaling.

The UPR is an adaptive response conventionally triggered by misfolded protein accumulation within ER lumen, which can be a consequence of various factors (Hypoxia, acidity etc.). It consists of three signaling branches driven by distinct sensors: IRE1, PERK and ATF6, all of them kept in their inactive form by chaperone protein GRP78. Upon damaged protein accumulation in ER, GRP78 binds with higher affinity to misfolded protein, which leads to UPR activation and signal transduction. IRE1 undergoes di/oligomerization, autophosphorylation and activation, cleaving XBP1 mRNA to its alternatively spliced sXBP1 form with potent transcription factor activity. Second ER sensor, PERK is also activated through dimerization and induces gene transcription through ATF4, at the same time modulating protein translation to alleviate stress of misfolded protein upon ER. The third sensor, ATF6 is translocated to the Golgi where it's cleaved to its transcriptionally active form. The resulting transcription factors drives expression of genes encoding chaperones, ERAD machinery and lipid molecules involved in lipid biosynthesis. Adapted from (Rubio-Patino, Bossowski, Chevet, & Ricci, 2018).

respectively. The activation of this branch is also required for UPR-dependent autophagy induction (I. Kim, Xu, & Reed, 2008).

The last UPR sensor to be mentioned is ATF6, which unlike the former two ER sensors does not undergo oligomeryzation, and instead is translocated to the Golgi apparatus upon GRP78 release, where it is cleaved by site-1 and site-2 proteases (S1P and S2P). The released part of this cleaved protein is a potent transcription factor which migrates to the nucleus triggering the expression of genes encoding chaperones, components of the ERAD machinery and proteins involved in lipid biochemistry (Hetz et al., 2015; I. Kim et al., 2008).

Therefore, the UPR appears to be one of a range of crucial mechanisms that is modified by cancer cells for their successful growth and spreading.

In the following chapter dualistic role of UPR activation and interactions between tumor and its microenvironment including immune involvement will be discussed. But first, we will look into some intriguing connection between UPR and aging, as many of the dietary regimens that suppress cancer growth have also been effective in delaying aging and increasing longevity, and their effect was proposed to be mechanistically obtained at least partially by enhanced proteostasis and UPR activity.

3. UPR and Aging

With age, tissues gradually lose the capacity to produce properly folded proteins, partially as a result of lower UPR activity and XBP1 in the most (Taylor & Dillin, 2013). Whole tissue over-expression of sXBP1 in *C. elegans* does not extend lifespan, despite increasing ER stress resistance.

Tissue specific sXBP1 expression revealed antagonistic effects on longevity, shortening the C. elegans lifespan when sXBP1 was overexpressed in muscle tissue, but extending it when overexpressed in neuronal or intestinal tissue (Taylor & Dillin, 2013). Thus, UPR modulation has positive effects on some tissues, while at the same time having negative effects on others. Interestingly, the neuronal activation of sXBP1 *per se* was physiologically irrelevant, suggesting that the nervous system adjusts the proteostasis machinery in the global, whole-organism manner, while the anti-aging effectors operate in the periphery, highlighting the intestine.

Protein misfolding is tightly connected with aging. In general, the older the animal is, the more damaged and misfolded peptide it accumulates. Thus, it could be a consequence of reduced UPR activity also observed in aged animal tissues. As UPR induces proteostasis i.e. via chaperone production, it has been proposed and shown that single heat shock protein (HSP) overexpression can extend lifespan in experimental model of *C. Elegans*, and multiple HSPs proved to have higher effect than single HSP induction (Swindell, 2009; Walker et al., 2001). That results point to hampered proteostasis as major factor in aging. Moreover, simpler organisms like yeast happen to express only one discovered ER stress sensor – IRE1, and it is a crucial protein in yeast lifespan extension triggered by CR (Choi, Kwon, & Lee, 2013; Labunskyy et al., 2014). Genetic approach experiments confirmed the effect of enhanced UPR and lifespan extension, as the reverse, where sXBP1 ablation reduced expected lifespan in *C. elegans* (Cui et al., 2015; Henis-Korenblit et al., 2010). More recently, essential vitamin D3 has been shown to reduce proteotoxicity in an aged *C. elegans* model, that resulted in extended lifespan, and was dependent on UPR components. More precisely, vitamin D3 treatment was able to specifically induce IRE1 activity (Mark et al., 2016).

4. UPR in Cancer

Cell types and tissues display very distinctive ER protein folding capacities, depending on their protein production demand and stress state. For example, during cancer transformation from healthy to malignant cells, there are many critical steps where internal (oncogene-driven protein synthesis) or external (nutrient shortages, hypoxia) factors impose high demand on protein synthesis and quality assessment in the ER (Cubillos-Ruiz et al., 2017; Hart et al., 2012). When the ER folding capacity is not sufficient to meet proper protein synthesis it results in ER stress and triggers a cascade of adaptive mechanisms named the UPR that is meant to restore proteostasis. Hence, the UPR will inhibit protein translation to halt the accumulation of misfolded protein, increase the production of proteins such as chaperones and foldases that assist in proper protein folding, and promote the degradation and elimination of misfolded proteins to limit their negative impact on cell physiology. The UPR induction serves as a cyto-protective and pro-survival mechanism, but when the ER stress cannot be restored and it prolongs, the same pathways that were initially engaged in cell survival, will lead to cell death, typically by apoptosis (M. Wang & Kaufman, 2014).

A. Persistent activation of UPR in cancer

In the TME cells encounter harsh conditions that are known to trigger cellular stress, such as low availability of oxygen and various nutrient, oxidative stress, tissue acidification, and improper lipid homeostasis (Cubillos-Ruiz et al., 2017). Deprivation of particular amino acids such as proline (Sahu et al., 2016) and glutamine (Shanware et al., 2014) has been shown to induce ER stress-dependent tumor growth inhibition and stimulates cytokine secretion, respectively. Finally, a high-caloric feeding before tumor onset induced unresolved ER stress in a KRAS-driven lung tumor model, leading to reduced tumor growth (Ramadori et al., 2015). These studies show that there is a direct connection between dietary, therefore metabolic modulation and the induction of the UPR. In addition, tumoral cells display considerably higher demand of protein synthesis at every step of cancer development, making them prone to chronic ER stress (Dejeans, Barroso, Fernandez-Zapico, Samali, & Chevet, 2015). As a matter of fact, spontaneous mouse and human lymphomas show higher levels of UPR activation when compared with normal tissues. In this context, it was shown that the oncogenes c-Myc and N-Myc activate the PERK/eIF2α/ATF4 pathway, which leads to cytoprotective-autophagy induction and decreased survival (Hart et al., 2012). In addition, the loss of tumor suppressor genes like p53 during transformation can induce the synthesis of proteins that were previously repressed, leading to ER stress induction (Namba et al., 2015).

The first description of the role of ER stress in cancer progression and sensitivity to chemotherapeutic agents was proposed almost 15 years ago (Y. Ma & Hendershot, 2004), and up to date many clinical studies have observed elevated expression of UPR actors such as IRE1α, XBP1, PERK and ATF6 in different types of cancers (Obacz et al., 2017). Importantly, the ER stress sensor GRP78 which serves as the key modulator of the UPR response has been found to facilitate cancer migration and its expression is positively correlated with the progression from early to advanced cancer stages (Niu et al., 2015; Tang et al., 2012; L. Zhang et al., 2017). GRP78 was also shown to control fatty acid oxidation and silencing of GRP78 resulted in increased drug sensitivity by modulation of lipid metabolism (Cook et al., 2016). Interestingly, an antibody targeting GRP78 has been shown to improve the efficacy of ionizing radiation therapy in human glioblastoma and non-small cell lung cancer cell lines *in vitro* and in athymic mice models (Dadey et al., 2017).

It is now clear that ER stress induction and consequent UPR activation are tightly linked and orchestrate many important features in cancer development and prognosis. How UPR regulation impacts on the different cancer types is still a subject of extensive research as the pathways involved in ER stress and their effect on the TME vary largely from one cancer type to another (Cubillos-Ruiz et al., 2017).

B. UPR and cancer hallmarks

The hallmarks of cancer proposed by Hanahan and Weinberg have been briefly discussed in chapter "Cancer – common denominator of many diseases", and here the particular focus on these hallmarks modulation and UPR will be presented (Hanahan & Weinberg, 2011). This list includes: resisting cell death, sustaining proliferative signaling, activating invasion and metastasis, enabling replicative immortality and inducing angiogenesis. Additionally, these traits are driven by cell genome instability, gene mutations and local inflammation, accompanied by modulation of metabolism and evasion of immune surveillance. Importantly, the UPR machinery regulates most if not all of these features.

- **Proliferative signaling**

Proliferation is usually halted upon UPR induction, as the adaptive UPR reduces protein synthesis (M. Wang & Kaufman, 2014). Indeed, XBP1 loss in intestinal stem cells increases their number *in vivo* and promotes tumor formation in the APC^{min} polyposis mice in an IRE1α-dependent manner (Niederreiter et al., 2013). Other studies have shown that induced ER stress impacts on a number of growth-promoting signaling pathways: p38 MAPK, PI3K, AKT/mTOR and Raf/MEK/ERK pathways (Garg, Maes, van Vliet, & Agostinis, 2015), and depending on the context, these pathways could promote or suppress cancer development (Darling & Cook, 2014).

- **Cell death evasion**

In order to evade death signals cancer cells can selectively induce the PERK/eIF2α/ATF4 arm of the UPR, which leads to increased cell survival by the induction of cytoprotective autophagy in Myc-induced tumor transformation (Hart et al., 2012). Autophagy is an intracellular degradation

process via delivery of cytoplasmic entities to the lysosomes, where macromolecules are lysed and their components recycled. Generally it is believed that defects in the autophagy machinery favors cancer initiation, while later on, the restoration of autophagic responses serve cancer cells as support for survival, proliferation and growth in the presence of harsh microenvironmental conditions (Galluzzi, Pietrocola, et al., 2015). Autophagy is induced in response to virus infection by the ATF6 and PERK branches of the UPR (M. Wang & Kaufman, 2014). Finally, the blockage of autophagy by knock-down of its regulator beclin 1 results in sensitization to tamoxifen upon sXBP1 overexpression (R. Hu et al., 2015).

- **Immortality**

Replicative immortality is obtained by cancer cells through activation of telomerase, ribonucleoprotein providing the main mechanism by which cells lengthen their telomeres that are shortened during cell division. It has been reported that telomerase undergoes activation due to the ER-stress dependent increased expression of the catalytic component of telomerase (TERT) (Zhou et al., 2014). That could be explained by upregulation of its transcriptional regulator β-catenin, which is induced by the UPR via the IRE1 branch (X. X. Li et al., 2017), but the direct link is still missing.

- **Genome instability**

Genome stability is affected by the similar threats that induce ER stress: ROS accumulation, hypoxia and heat stress as the first examples. Indeed, tunicamycin-induced ER stress and glucose deprivation affects the induction of the mechanisms of DNA repair via proteasomal degradation of Rad51, the protein engaged in DNA double strand break reparation (Yamamori, Meike, Nagane, Yasui, & Inanami, 2013). Additionally, there is reason to believe that hypoxia can lead to aberrant DNA repair, genomic instability, and results in cellular cancer transformation (Bristow & Hill, 2008). Finally, increased GRP78 transcription from ER stress can also facilitate DNA damage repair through recruitment of arginine histone methyltransferase PRMT1, and ER stress could favor histone H4 acetylation, known to be involved in the chromatin structure regulation and transcription factor recruitment (Baumeister et al., 2005).

- **Metastasis**

Metastatic outgrowth refers to cancer cell migration from its primary tumor site and colonization of secondary tissues, very often distant and having a substantially different microenvironment. Clinically, metastasis is a very negative prognostic indicator, correlated with high mortality. The UPR contributes to this process by facilitating extracellular matrix protein production to enable cell migration and invasion. Firstly, the PERK branch of the UPR facilitates cancer cell survival in response to the loss of cell-to-cell contact, as it is required to perform epithelial-to-mesenchymal transition (Dey et al., 2015; Feng et al., 2014). Secondly, silencing the PERK/ATF4 pathway by chemical inhibition reduces metastasis *in vivo* in NOD/SCID models of breast cancer metastasis (Dey et al., 2015; Feng et al., 2014), possibly by blocking the upregulation of LAMP3, protein involved in enhanced cell migration and metastatic abilities (Mujcic et al., 2013). Finally, tumor dormancy, a critical step for invasive cancer cells to implant in a pre-metastatic niche, has been shown to be dependent on p38 activation which drives GRP78 upregulation and PERK phosphorylation (Ranganathan, Zhang, Adam, & Aguirre-Ghiso, 2006) as well as ATF6 activation (Cubillos-Ruiz et al., 2017; Schewe & Aguirre-Ghiso, 2008). UPR components can control cell cycle and proliferation rates by IRE1 and PERK, thus contributing to tumor dormancy and chemotherapy resistance (Urra, Dufey, Avril, Chevet, & Hetz, 2016). This ability of anti-proliferative dormancy enables cancer cells to survive the initial phase of establishment in foreign environments and to become proliferatively active when they adapt to new conditions, hence promoting cancer recurrence.

- **Metabolism deregulation**

Deregulated cellular energetics can be also shaped by UPR. The mitochondrial-associated membrane (MAM) and the ER can spatially interact, modulating apoptosis and mitochondrial fission/fussion dynamics (Hetz et al., 2015). These interactions are possible as least partially by mitofusin 2 (Mfn2), a GTPase protein localized in the MAM. It has been shown that Mfn2 physically interacts with PERK, regulating mitochondrial–ER interactions, thus impacting on metabolism, calcium signaling and apoptosis (Munoz et al., 2013). Mfn2 ablation leads to a potent ER stress response, at the same time reducing apoptosis and autophagy (W. Wang et al., 2015). In

addition, sXBP1 was found to drive phospholipid biosynthesis and secretory pathway gene expression, supporting ER expansion (Sriburi et al., 2007).

As described above, by inducing a specific sub-lethal manner of the UPR machinery cancer cells obtain multiple benefits, enabling them to outcompete healthy cells, escape intrinsic and extrinsic anti-tumoral mechanisms and colonize new physiological niches where they can thrive and progress. Many of these adaptations result from close links of ER stress with UPR-derived autophagy and mitochondrial crosstalk. Understanding how and in which manner cancer cells hijack UPR machinery could open new perspectives and improved and effective anti-cancer therapies.

C. UPR and cell death regulation

The UPR serves to restore proteostasis, but if unresolved, the same pathways that were initially used for survival are leading to cell death, primarily by apoptosis (**Figure 15**). For example, under acute ER stress the PERK/eIF2α/ATF4 pathway leads to the inhibition of protein translation resulting in cell survival. However, prolonged PERK activation induces signals leading to the translation of ATF4 that regulates CHOP induction and results in apoptosis (Pytel, Majsterek, & Diehl, 2016).

Perturbation of calcium homeostasis in the ER is central in UPR death pathways signaling. ER stressors brefeldin A and tunicamycin lead to cytosolic calcium ions accumulation, which triggers cell death through caspase-12-dependent apoptosis in neuronal cells. Interestingly, pharmacological activation of SK2 channels protects against cell death induced by these compounds, pointing towards calcium homeostasis as the critical event in cell death induction upon ER stress (Richter et al., 2016). Moreover, calcium homeostasis regulates cell fate through the BCL-2 family of proteins, many of which reside within the ER. The UPR directly regulates the activity of these pro- and anti-apoptotic proteins by modulating their ratio. Interestingly, the anti-apoptotic proteins BCL-2 and BCL-X_L physically associate with the inositol trisphosphate receptor and alter its ability to release calcium ions lowering the ER calcium basal levels (Vervliet, Parys, & Bultynck, 2016), whereas the pro-apoptotic protein BAX has the opposite effect (R. G. Jones et al., 2007). Another example of how the UPR controls BCL-2 family members is the activation of

IRE1α, which leads to the inhibition of BCL-2 and induces the phosphorylation of the pro-apoptotic protein BIM via binding to JNK and TNF receptor-associated factor 2 (TRAF2), favoring apoptosis induction (M. Wang & Kaufman, 2014). Moreover, the pro-apoptotic proteins PUMA and BIM are transcriptionally induced by CHOP in response to ER stress (Ghosh, Klocke, Ballestas, & Roth, 2012; Puthalakath et al., 2007), whereas CHOP represses BCL-2 expression (M. Wang & Kaufman, 2014). Finally, CHOP and ATF4 mRNAs and proteins have short half-lives explaining why sustained activation of the UPR pathways is necessary to induce cell death (M. Wang & Kaufman, 2014).

Even though BIM has been found to be essential to induce ER stress-mediated apoptosis in a range of cell types both *in vitro* and *in vivo* (Puthalakath et al., 2007), the other BH3-only proteins such as BID, NOXA and PUMA appear to have only partial effect (Hetz et al., 2015). BOK (BCL-2 Ovarian Killer) has been recently described to be a unique regulator of apoptosis activation under ER-stress conditions, independently of BAX and BAK expression, connecting apoptotic signals at the ER membrane to apoptotic induction in the mitochondria (Carpio et al., 2015).

More interestingly, BOK is not activated by the BH3-only proteins or inhibited by the antiapoptotic proteins. In fact, BOK is constitutively degraded via the ERAD pathways, being stabilized when the proteasome is inhibited (Llambi et al., 2016). Additionally, the UPR-mediated inhibition of protein translation decreases Mcl-1 protein levels, important antiapoptotic member of the BCL-2 family protein, therefore sensitizing resistant myeloma cells to apoptosis induced by thapsigargin (Gomez-Bougie, Halliez, Moreau, Pellat-Deceunynck, & Amiot, 2016).

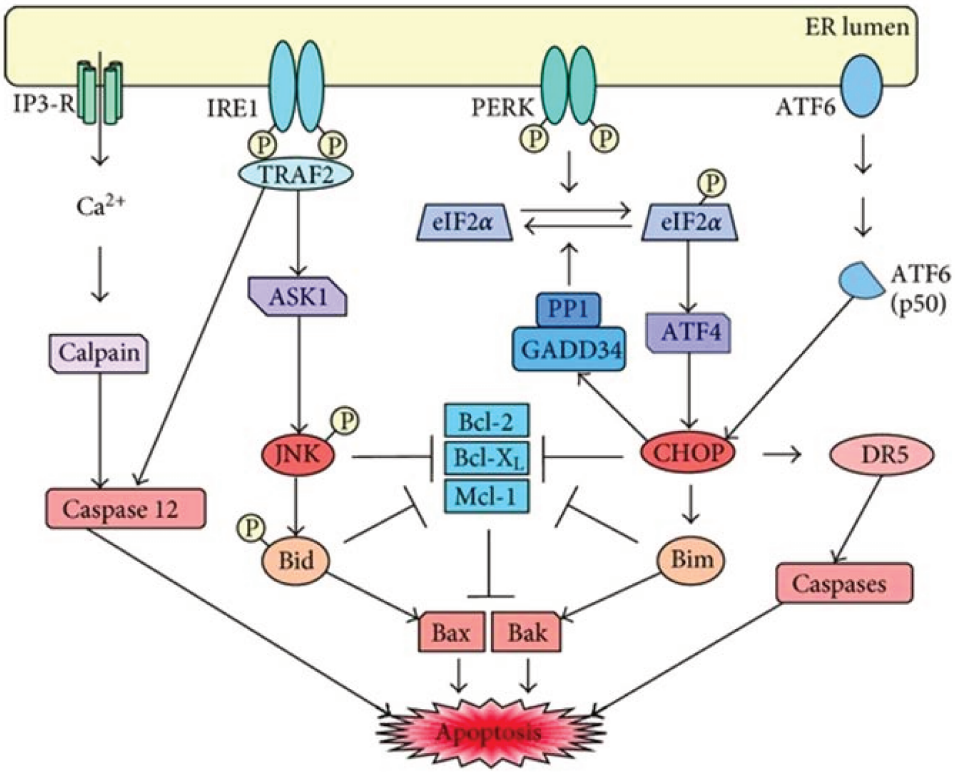


Figure 15: ER stress as a driver of apoptosis.

Non-resolved persistent ER stress leads to increased ration of pro-apoptotic proteins Bim, Bid, Bax and Bak over anti-apoptotic Bcl-2 family. Transcription factors ATF4 and ATF6 stimulate CHOP expression. CHOP inhibits proteins of the Bcl-2 family and stimulates pro-apoptotic Bim, favouring heterodimerization and activation of Bax and Bak. CHOP also induce expression of DR5 - cell surface death receptor. JNK phosphorylation by protein kinase ASK1 leads to Bid activation. Association of TRAF2 with IRE1 also leads to activation of caspase 12. Calcium release from the ER via IP3 receptors result in caspase 12 activation via Calpain activation. Adapted from (Schonthal, 2012).

IRE1α has an interesting dual role in cell death regulation owing to its kinase and endoribonuclease activity (Han et al., 2009; Maurel, Chevet, Tavernier, & Gerlo, 2014). In recent years there has been tremendous advancement in understanding IRE1α signaling. Firstly, it has been shown that the oligomeryzation state of IRE1α determines its activity towards either RIDD or XBP1 splicing. The oligomeryzation state will favor splicing of XBP1 to sXBP1, whereas the monomer/dimer form exhibits mostly mRNA cleavage activity by RIDD (Tam, Koong, & Niwa, 2014). Secondly, the inhibition of the kinase activity of IRE1α has been shown to bypass its autophosphorylation

and to activate the endoribonuclease activity by an alternate mode that enforces XBP1 splicing and limits mRNA decay and apoptosis (Han et al., 2009). *In vivo* IRE1α facilitates tumor growth by promoting the expression of the tumorigenesis driver β-catenin, and IRE1α chemical inhibition or genetic knock-down inhibited colonic tumorigenesis in an immunodeficient mouse model, confirming its pro-survival role (X. X. Li et al., 2017). Altogether, the activation of IRE1α and its effect on cancer cell death or survival seems to be highly dependent on its oligomeryzation state which modulates its endoribonuclease and kinase activities.

ATF6 activation through ER-stress induction leads to the increased expression of genes involved in UPR and ERAD: XBP1, GRP78, chaperones and oxidoreductases, among others. Its role in the UPR is majorly cytoprotective, and its activation contributes to fine-tuning of the IRE1α and PERK signaling, eliciting pro-survival properties *in vitro* and *in vivo* (Cubillos-Ruiz et al., 2017; I. Kim et al., 2008; Schewe & Aguirre-Ghiso, 2008). ATF6 activation has been linked to increased tumor dormancy and increased tumor resistance to chemotherapy, interestingly by AKT-independent activation of mTOR signaling (Schewe & Aguirre-Ghiso, 2008).

Apoptosis is known as a tolerogenic type of cell death because, up to date, it was believed that it did not induce an immune response. However, some forms of cell death produced by certain ER stress-inducing stimuli lead to the release of danger signals by the dying cell, indicating that cell death signaling under ER stress could be even more complex and cell type-/context- dependent (discussed below) (Galluzzi et al., 2017a).

Taken together, cancer cells undergoing ER stress will induce the UPR, which in a context and time dependent manner will favor pro-survival or pro-death pathways resulting in enhanced or decreased tumor progression.

5. Endoplasmic reticulum and immunogenic cell death

There is growing evidence that the UPR can control the anti-tumor immune response by modulating the character of cancer cell death. The UPR can regulate the release DAMPs it was already defined, which are classical “find me” and “eat me” signals. In brief, DAMPs are intracellular molecules, usually ubiquitous and tightly regulated, that are hidden from the immune system’s recognition

under normal conditions. However, upon acute stress or death induction, cells can induce an immunogenic-driver response by the expression of DAMPs on the cell surface (e.g. CRT and HSPs) or by their extracellular release (e.g. ATP and High Mobility Group Box 1 - HMGB1) (Galluzzi et al., 2017a; Grootjans, Kaser, Kaufman, & Blumberg, 2016). This type of cell death is known as Immunogenic Cell Death (ICD) (**Figure 16**). Interestingly, ICD must be preceded by the ER stress and UPR in order to induce CRT and HSPs surface exposure. In the case of CRT exposure it has been suggested that this event depends on activation of the PERK/eIF2α pathway (Panaretakis et al., 2009). Furthermore, ATP release depends on induction of pre-mortem autophagy (Galluzzi et al., 2017a). The importance of ATP and HMGB1 release can be appreciated in the $Ripk3^{-/-}$ or $Mlkl^{-/-}$ TC-1 syngeneic mouse lung cancer cells, which are impaired in secretion of these two DAMPs under canonical ICD inducer Mitoxantrone. As opposite to their WT counterparts, $Ripk3^{-/-}$ or $Mlkl^{-/-}$ TC-1 fails to induce immune response, which can be rescued by local administration of ATPases plus a synthetic TLR4 ligand, where the by APCs and $CD8^+$ T cells intra-tumoral infiltration is restored (H. Yang et al., 2016).

CRT binds CD91, ATP binds purinergic receptors (P2Y2 or P2X7) and HMGB1 binds TLR4, respectively (Galluzzi et al., 2017a). These receptors are found on DCs and promote engulfment of dying cells, attraction of dendritic cells into the tumor bed, production of IL-1β and tumor antigen presentation, respectively. In the case of CRT, it is a highly conserved calcium-binding ER chaperone that has important functions in the immune response. For example, CRT is a chaperone for MHC class I molecules, regulating antigen presentation hence affecting recognition by $CD8^+$ T cells (Raghavan, Wijeyesakere, Peters, & Del Cid, 2013). It is also associated with the increased expression of CD86, CD80 and MHC class II in the cell surface of DCs, leading to an efficient anti-cancer $CD8^+$ T cell response (Grootjans et al., 2016). CRT exposure also plays an important role in the immunosurveillance mechanism induced by cells

that have increased ploidy (Senovilla et al., 2012). Even though we know that ER stress induces CRT exposure to the cell surface during ICD, the mechanism by which this phenomenon happens is still unknown.

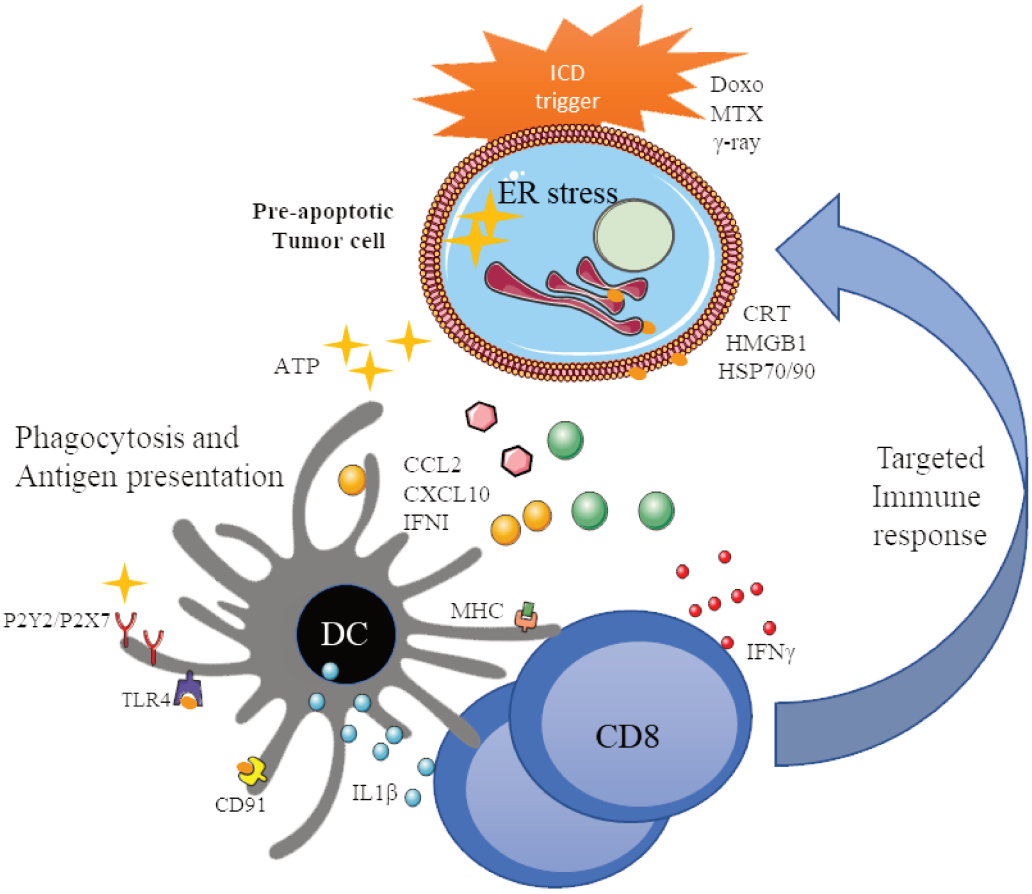


Figure 16: ICD and immune activation.

Immunogenic cell death (ICD) is an active process of exposing and secreting DAMPs in order to attract immune cells and elicit immune response. Canonical ICD inducers sach as Doxorubicin (Doxo), Mitoxantrone (MTX) and gamma-irradiation (γ-ray) triggers Calreticuline (CRT), High Mobility Group Box 1 (HMGB1) and some of the heat shock protein family (HSP) exposure as an „eat me" signals. As a result, professional phagocytic cells like dendritic cells (DCs) are activated and through antigen presentation and cytokine stimulation programme $CD8^+$ T cells in targeted cytotoxic response toward malignant cells.

HMGB1 secretion during cell death can activate the UPR in DCs by increasing GRP78 expression and XBP1 splicing. When XBP1 was silenced, there was a downregulation of CD86 and CD80 cell

surface activation markers and MHC class II expression. These events resulted in the decrease of T cell proliferation and differentiation affecting the activation of T cells in *ex vivo* co-cultures (Grootjans et al., 2016). In more recent studies, increased expression of HMGB1, HMGN1, XBP1 and p-eIF2α was correlated with a high amount of tumor infiltrating lymphocytes in triple negative breast cancer (TNBC) patients (Park et al., 2016).

Besides DAMPs, there are also "don't eat me" signals that will help cancer cells avoid the immune system's recognition. ER stress regulated proteins also control these signals. For example, GRP78 inhibition in BALB/c and athymic tumor-bearing mice increased Monocyte Chemoattractant Protein-1 (MCP-1) serum levels and regulated CD47, a glycoprotein of the immunoglobulin superfamily critical in self-recognition. Non-malignant tissue increased the CD47 "don't eat me" signal in response to GRP78 inhibition, while the tumoral tissue decreased its expression. In this way, GRP78 inhibition stimulated macrophage infiltration and reduction of estrogen receptor–positive breast cancers (Cook et al., 2016).

In summary, the UPR has a very important role in ICD induction and constitutes a promising target for the development of anti-cancer strategies. This is the case for checkpoint blockade immunotherapies that can only benefit patients with tumors that have TILs previous to the treatment. Tumors that do not have TILs can be sensitized to checkpoint blockade immunotherapies when combined with ICD-inducing drugs (Pfirschke et al., 2016). In this sense, the co-administration of chemotherapies that do not induce ICD with immunogenic chemotherapies capable of inducing the UPR should be considered as a promising anti-cancer strategy.

6. UPR cross-talk and immune regulation

A. NFκB

The UPR can crosstalk with different signaling pathways in order to regulate tumor-host interactions. Interestingly, the three branches of the UPR have been shown to induce the pro-inflammatory NF-κB pathway. IRE1 interacts with TRAF2, recruiting IκB kinase (IKK) and inducing the phosphorylation and degradation of IκB, which allows NFκB to translocate to the nucleus and regulate the transcription of its target genes. IκB has a shorter half-life than NFκB and for this reason, changes in protein translation under ER stress stimuli that activate the PERK pathway, will induce the NFκB pathway by affecting the IκB:NFκB ratio. Finally, ATF6 can induce NFκB through the activating phosphorylation of AKT (Grootjans et al., 2016).

B. Hypoxia

Under low oxygen availability, the UPR can interact with the hypoxia HIF-1α pathway, which can promote vascularization, glycolysis, and survival. When hypoxic conditions are combined with ER stress inducers the HIF1α pathway and the UPR synergize to activate downstream targets, including VEGF (Pereira, Frudd, Awad, & Hendershot, 2014). An example of this synergy is when HIF1α, which is hyperactivated in TNBC, heterodimerizes with sXBP1 under hypoxic conditions, correlating with poor patient prognosis. They will both function as co-regulators and regulate the transcription of HIF-1α transcriptional targets (i.e. *VEGF-A, PDK1, GLUT1* and *DDIT4*) (Chen et al., 2014). Control of mRNA translation is an important cellular response to both ER stress and hypoxia. Hypoxia activates the PERK/eIF2α/ATF4 pathway leading to the inhibition of global mRNA translation. The translation of ATF4 will still take place in a HIF1α-independent but PERK/eIF2α/ATF4-dependent manner, most likely through Siah proteins. Siah1 and Siah2 are proteins that stabilize HIF1α and ATF4, through prolyl hydroxylase 1 (PHD1) downregulation. The PERK/ATF4 and IRE1α/xBP1 pathways can also induce Siah2. The fact that Siah2 has sXBP1 responsive elements in its promoter raises the possibility of a direct control of the PERK/ATF4 pathway by the IRE1 pathway. This could be the first step in the response to stress conditions as Siah1 and Siah2 can stabilize HIF1α and then it is possible that HIF1α could replace sXBP1 in the

Siah promoter, as their response elements overlap (Scortegagna et al., 2014). The PERK/eIF2α/ATF4 branch of the UPR has been shown to have a pro-survival effect on tumor cells under hypoxic conditions. This occurs through the UPR-mediated activation of pro-survival autophagy and its inhibition increases cell death (M. Wang & Kaufman, 2014).

C. Response to pathogen

The similarities between the antigen-specific immune response triggered by ICD and the one induced by pathogen infection have led scientists to investigate these pathways commonalities, in hope to apply this knowledge in cancer research. This is the case of TLRs, which are pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs). Activation of TLRs and the IRE1α/sXBP1 pathway are interconnected and result in the induction of the innate immune surveillance in response to pathogen infection. In macrophages, TLR activation will induce a ROS-dependent specific activation of the IRE1α/sXBP1 pathway, but not of the other arms of the UPR. Then, sXBP1 will induce IL-6 and IFN-β cytokine production (Martinon, Chen, Lee, & Glimcher, 2010). This kind of response is not restricted to TLRs, as there is a clear link between the UPR and Retinoic acid-inducible gene-I (RIG-I)-like Receptors (RLRs). RLRs are RNA helicases that sense pathogenic RNA and initiate antiviral immunity. Recent studies have linked IRE1α with the RIG-I pathway upon pathogen infection (Cho et al., 2013) and pathological conditions (Eckard et al., 2014). Upon the activation of IRE1α's endonuclease activity, the cleavage of endogenous RNA through its downstream pathway RIDD (Regulated IRE1-Dependent Decay) may produce fragments that resemble those of pathogens as they lack 5'-caps or 3'-polyA-tails that mark mRNA as "self". These fragments will activate RIG-I that will induce an innate immune response.

D. Endogenous RNA sensing

In the context of cancer, endogenous RNAs that are not shielded by RNA binding proteins have already been shown to act as DAMPs for PRRs. It was observed that in primary human breast cancers, activated stromal cells present unshielded RNA in exosomes in order to propagate anti-viral signaling to the tumor microenvironment. These unshielded RNAs in stromal exosomes result

in an inflammatory response when transferred to immune cells and in tumor growth and invasion when transferred to breast cancer cells (Nabet et al., 2017). These studies were performed *in vitro* and in athymic mice, but other studies performed in immunocompetent mice demonstrated that RIG-I activation induced the secretion of extracellular vesicles by melanoma cells that act as immune activating agents favoring the anticancer immune response (Dassler-Plenker et al., 2016). Studies in immunocompetent mice demonstrated that the administration of a siRNA designed to silence Bcl-2 and activate RIG-I efficiently inhibited tumor growth through an antitumor immune response. This antitumor response involved the activation of myeloid and plasmacytoid DCs, NK cells, $CD4^+$ and $CD8^+$ T cells and was associated with the secretion of type I cytokines (IFNα, IL-12p40 and IFNγ) (Poeck et al., 2008). Furthermore, RIG-I has been proposed as a tumor suppressor in HCC as RIG-I deficiency promotes hepatocellular carcinoma (HCC) carcinogenesis (Hou et al., 2014). Other studies in highly immunodeficient mice have suggested that RIG-I can inhibit tumor growth by inducing apoptosis through the regulation of BH3-only proteins (Besch et al., 2009). Additionally, it was described that pancreatic cancer cells treated with RIG-I–like helicase ligands die through immunogenic cell death (ICD). This ICD occurred through the translocation of CRT to the cell surface and the posterior release of HMGB1 that activated DCs and cytotoxic $CD8^+$ T cells (Duewell et al., 2014).

E. Proinflammatory cytokines and interferons

The link between UPR and IFNI was not clear, as UPR inducing agents (tunicamycin and thapsigargin) have not been found to trigger any production or secretion of type I IFNs (J. A. Smith et al., 2008). However, combined with LPS or poly I:C (agonists of TLR4 and TLR3) the increase in IFNI was massively induced as compared to PRR agonist alone (F. Hu et al., 2011; J. A. Smith et al., 2008). In addition, other pro-inflammatory cytokines including IL-6 and TNFα have been induced as well, revealing more general UPR control over cytokine secretion (Martinon et al., 2010; J. A. Smith et al., 2008).

IRE1 RNase chemical inhibition was recently shown to sensitize breast cancer tumors to paclitaxel treatment and prolong survival of TNBC tumor-bearing immunodeficient mice (Logue et al., 2018). Mechanistically, IRE1 promoted secretion of pro-inflammatory cytokines (such as CXCL1,

IL-6 and IL-8) which in turn enhanced cell proliferation and growth. This result suggest that IRE1 activity would accelerate tumor growth, which was not the case when tested *in vivo* upon IRE1 RNase chemical inhibition. Nevertheless, this finding strengthen the idea of IRE1 functions as the master regulator of cell the secretome. Although, as cell secretome varies from one to another cell type it is rational to assume that depending on cell type where IRE1 is modulated one can expect different, possibly even contradictive outcomes of that regulation.

The pro-tumorigenic or antitumorigenic effects of individual cytokines are context dependent and heavily influenced by synergisms in the complex cytokine milieu. UPR, as central actor in the cell secretome control, plays crucial part in cytokine modulation, and here we discuss some of the cytokine possibly affected by UPR and implicated in tumor development.

Altogether, these studies highlight the importance of studying the regulation of the UPR in the context of cancer in order to understand immunogenicity and to improve the antitumor immune responses and therapies.

Results

1. Article 1

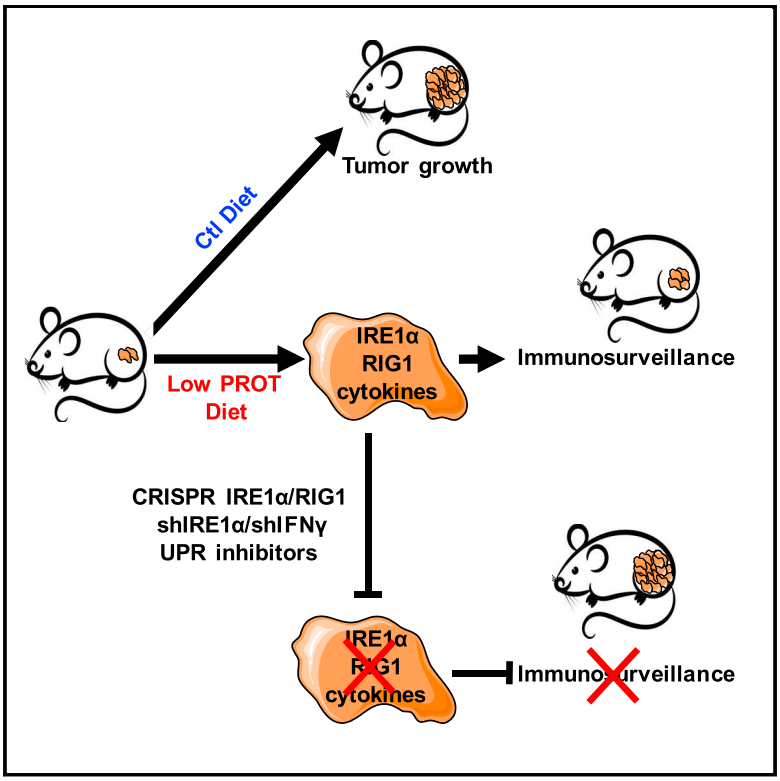
*Rubio-Patino, C., ***Bossowski, J. P**., De Donatis, G. M., Mondragon, L., Villa, E., Aira, L. E., Chiche, J., Mhaidly, R., Lebeaupin, C., Marchetti, S., Voutetakis, K., Chatziioannou, A., Castelli, F. A., Lamourette, P., Chu-Van, E., Fenaille, F., Avril, T., Passeron, T., Patterson, J. B., Verhoeyen, E., Bailly-Maitre, B., Chevet, E. & Ricci, J. E. (2018) Low-Protein Diet Induces IRE1alpha-Dependent Anticancer Immunosurveillance, Cell Metab. 27, 828-842 e7

Article

Cell Metabolism

Low-Protein Diet Induces IRE1α-Dependent Anticancer Immunosurveillance

Graphical Abstract



Authors

Camila Rubio-Patiño,
Jozef P. Bossowski,
Gian Marco De Donatis, ...,
Béatrice Bailly-Maitre, Eric Chevet,
Jean-Ehrland Ricci

Correspondence

ricci@unice.fr

In Brief

Dietary restriction (DR) slows down tumor growth by increasing tumor immunosurveillance. Rubio-Patiño et al. show that a moderate reduction in dietary protein intake, rather than carbohydrate reduction, without overall calorie changes, activates the IRE1α/RIG1 pathway in tumor cells resulting in an anticancer immune response in mice.

Highlights

- Feeding mice with a diet presenting a decrease in protein limits tumor growth
- Low PROT diet increases tumor-infiltrating lymphocytes
- Feeding mice with a Low PROT diet induces the IRE1α/RIG1 pathway in tumor cells
- IRE1α/RIG1 activation in tumor cells results in an anticancer immune response

Rubio-Patiño et al., 2018, Cell Metabolism 27, 828–842
April 3, 2018
https://doi.org/10.1016/j.cmet.2018.02.009

CellPress

CellPress

Cell Metabolism
Article

Low-Protein Diet Induces IRE1α-Dependent Anticancer Immunosurveillance

Camila Rubio-Patiño,[1,9] Jozef P. Bossowski,[1,9] Gian Marco De Donatis,[1] Laura Mondragón,[1] Elodie Villa,[1] Lazaro E. Aira,[1] Johanna Chiche,[1] Rana Mhaidly,[1] Cynthia Lebeaupin,[1] Sandrine Marchetti,[1] Konstantinos Voutetakis,[2,3] Aristotelis Chatziioannou,[2,4] Florence A. Castelli,[5] Patricia Lamourette,[5] Emeline Chu-Van,[5] François Fenaille,[5] Tony Avril,[6,7] Thierry Passeron,[1] John B. Patterson,[8] Els Verhoeyen,[1] Béatrice Bailly-Maitre,[1] Eric Chevet,[6,7] and Jean-Ehrland Ricci[1,10,*]

[1]Université Côte d'Azur, INSERM U1065, C3M, 151 route de St Antoine de Ginestière, BP 23194, 06204 Nice, France
[2]Institute of Biology, Medicinal Chemistry and Biotechnology, NHRF, 11635 Athens, Greece
[3]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece
[4]e-NIOS Applications PC, 17671 Kallithea-Athens, Greece
[5]Service de Pharmacologie et Immunoanalyse (SPI), Laboratoire d'Etude du Métabolisme des Médicaments, CEA, INRA, Université Paris Saclay, MetaboHUB, 91191 Gif-sur-Yvette, France
[6]Inserm U1242 «Chemistry, Oncogenesis, Stress, Signaling», Université Rennes 1, 35042 Rennes, France
[7]Centre de Lutte Contre le Cancer Eugène Marquis, 35042 Rennes, France
[8]Fosun Orinove, Newbury Park, CA 91320, USA
[9]These authors contributed equally
[10]Lead Contact
*Correspondence: ricci@unice.fr
https://doi.org/10.1016/j.cmet.2018.02.009

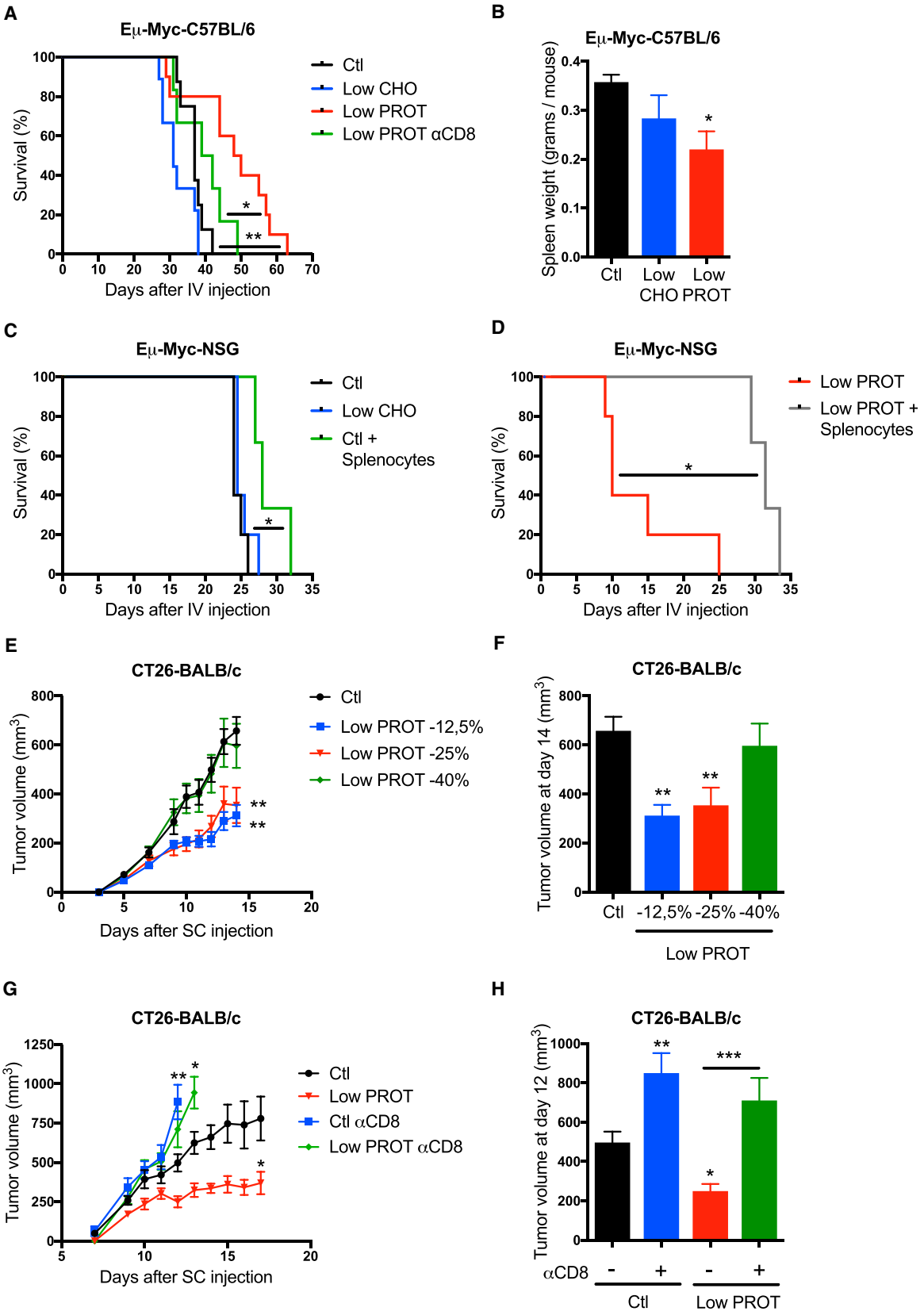
SUMMARY

Dietary restriction (DR) was shown to impact on tumor growth with very variable effects depending on the cancer type. However, how DR limits cancer progression remains largely unknown. Here, we demonstrate that feeding mice a low-protein (Low PROT) isocaloric diet but not a low-carbohydrate (Low CHO) diet reduced tumor growth in three independent mouse cancer models. Surprisingly, this effect relies on anticancer immunosurveillance, as depleting $CD8^+$ T cells, antigen-presenting cells (APCs), or using immunodeficient mice prevented the beneficial effect of the diet. Mechanistically, we established that a Low PROT diet induces the unfolded protein response (UPR) in tumor cells through the activation of IRE1α and RIG1 signaling, thereby resulting in cytokine production and mounting an efficient anticancer immune response. Collectively, our data suggest that a Low PROT diet induces an IRE1α-dependent UPR in cancer cells, enhancing a CD8-mediated T cell response against tumors.

INTRODUCTION

Dietary restriction (DR) without malnutrition, which includes caloric restriction (CR) and fasting, is well recognized as one of the most reliable methods to enhance life span and reduce the incidence of a wide variety of diseases, including human cancers (Fontana and Partridge, 2015). DR has a beneficial impact on health and life span, affects cancer development (Longo and Mattson, 2014; Lu et al., 2017; Meynet and Ricci, 2014), and sensitizes tumor cells to chemotherapy (Meynet et al., 2013; Rubio-Patiño et al., 2016), notably by increasing tumor immunosurveillance (Di Biase et al., 2016). The identification of new methods to induce immunosurveillance has become crucial for the development of effective therapeutic protocols against cancers, the inhibition of tumor development and progression, and the enhancement of long-term protection. However, using DR interventions, such as acute fasting or prolonged CR, to reduce tumor growth can be very difficult to tolerate for most cancer patients receiving treatment due to the development of cachexia and DR-related weight loss (Porporato, 2016). Therefore, developing alternative methods to benefit from DR-mediated reduction in tumor growth without impacting total caloric intake is an area of intense research (Bénéteau et al., 2012; Pietrocola et al., 2016). Indeed, it has been suggested that macronutrient modulation rather than calorie intake determines the effect of DR on health and aging (Levine et al., 2014; Solon-Biet et al., 2014). Interestingly, several studies have demonstrated that the balance of protein in the diet is especially important for these effects, as a low-protein (Low PROT) diet produces beneficial metabolic effects similar to DR, such as reductions in insulin-like growth factor 1 (IGF-1) and cancer incidence and an increase in longevity (Fontana et al., 2013; Levine et al., 2014; Solon-Biet et al., 2014, 2015).

Macronutrient modulation might also impact protein homeostasis, which is also referred to as proteostasis, and in particular endoplasmic reticulum (ER) functions (Cubillos-Ruiz et al., 2017). Consequently, the adaptive unfolded protein response (UPR) is stimulated when ER proteostasis is disturbed. Accumulating evidence indicates that chronic activation of the UPR supports the main hallmarks of cancer by favoring cancer cell autonomous and non-autonomous processes, which ultimately condition the immunosuppressive and pro-tumorigenic microenvironment (Cubillos-Ruiz et al., 2017). However, certain forms of ER stress



(legend on next page)

(environmentally or therapy-induced) can elicit immunogenic cancer cell death (ICD), which enables the release of key immunostimulatory (such as interferon γ [INFγ]) and danger signals, eventually driving efficient antitumor immunity (Galluzzi et al., 2015; Garg et al., 2015).

The UPR is controlled by three main ER resident sensors: Inositol Requiring Enzyme 1α (IRE1α), Activating Transcription Factor 6α (ATF6α), and PKR-like endoplasmic reticulum kinase (PERK) (Hetz et al., 2015). Upon ER stress, IRE1α oligomerizes and auto-transphosphorylates, thus activating the endoribonuclease domain that subsequently catalyzes the non-conventional splicing of X-box binding protein 1 (XBP1) (Calfon et al., 2002; Lee et al., 2002) together with the tRNA ligase RtcB (Lu et al., 2014). Spliced XBP1 (sXBP1) is an active transcription factor that regulates the expression of genes encoding proteins involved in protein folding and quality control, ER-associated degradation, and phospholipid synthesis. IRE1α RNase activity is also involved in regulated IRE1-dependent decay (RIDD) of mRNA, rRNA, and microRNAs (Maurel et al., 2014). Interestingly, in response to ER stress, RIDD-mediated RNA cleavage into single-strand fragments lacking markers of self was shown to activate RIG1 (Retinoic Acid Inducible Gene 1). This RIDD-RIG1 pathway affects in turn adaptive immunity in humans (Eckard et al., 2014). Similar to IRE1α, on ER stress, PERK dimerizes and trans-autophosphorylates, thus leading to the phosphorylation of the translation initiation factor eIF2α and global protein synthesis attenuation. This also leads to translational activation of ATF4, a transcription factor that controls the expression of genes whose products are involved in amino acid transport, autophagy, and redox control (Pakos-Zebrucka et al., 2016). Finally, ER stress induces ATF6 export from the ER and its transmembrane cleavage by Site-1 protease (S1P) and Site-2 protease (S2P), two Golgi resident proteases. The released cytosolic ATF6 fragment acts as an active transcription factor (Hetz et al., 2015).

Based on these premises, we analyzed the mechanism by which macronutrient modulation can mimic the antitumoral properties of DR by feeding mice *ad libitum* with isocaloric custom diets. Our results demonstrate that an isocaloric Low PROT diet but not a low-carbohydrate (Low CHO) diet can induce an IRE1α/RIG1-dependent increase in immunosurveillance, suggesting that such a diet can represent a clinically interesting alternative to DR interventions in the context of cancer development, tumor immunity, and treatment.

RESULTS

A Low-Protein Diet Limits Tumor Development through the Induction of Immunosurveillance

To analyze the effect of macronutrient modulation on tumor development, we fed mice bearing myc-driven lymphoma isolated from Eμ-Myc mice (Adams et al., 1985) custom engineered isocaloric diets containing either 25% less proteins (Low PROT) or 25% less carbohydrates (Low CHO) compared with the control diet. Mice were fed *ad libitum* with the different diets, and the regimens' impact on mouse survival was monitored over time (Figure 1A). We established that only Low PROT diet increased mouse survival, whereas global food intake and mouse weight were equivalent for all diets (Figures S1A and S1B). The increase in survival observed under the Low PROT diet was associated with a reduction of spleen weight compared with the other groups, thus indicating reduced lymphoma development (Figure 1B). Moreover, lymphoma-bearing lymph nodes from immunocompetent mice fed with a Low PROT diet presented an increase in *IFNγ* expression (Figure S1C). Given that IFNγ is a key cytokine for innate and adaptive immunity, we hypothesized that the Low PROT-dependent increase in survival could, at least in part, be due to an enhanced anticancer immune response. To test this hypothesis, we depleted cytotoxic $CD8^+$ T cells using an anti-CD8-specific antibody (Figures S1D and S1I). Strikingly, the impairment of the cytotoxic immune response prevented the beneficial effect induced by the Low PROT diet, suggesting a central role for $CD8^+$ T cells (Figure 1A).

To further confirm the role of Low PROT diet-induced immunosurveillance, we transplanted primary lymphoma cells isolated from Eμ-Myc mice into immunodeficient NOD-Scid gamma $c^{-/-}$ (NSG) mice. As shown in Figure 1A, a Low CHO diet did not impact mouse survival compared with the control diet (Figure 1C). Conversely, a Low PROT diet led to a dramatic decrease in mouse survival compared with the control diet (Figure 1D). Importantly, the co-injection of C57BL/6 splenocytes (containing mainly T and B cells syngeneic with the Eμ-Myc lymphoma cells injected) resulted in an increase in mouse survival under a Low PROT diet (Figure 1D). These findings further confirm the key role of the immune system in the beneficial effect of the Low PROT diet on tumor development.

We extended our observations *in vivo* using two additional mouse models: a murine colorectal carcinoma (CRC) cell line (CT26) subcutaneously injected into syngeneic

Figure 1. A Low PROT Diet Affects Tumor Development in a CD8-Dependent Manner

(A) Survival curve of syngeneic C57BL/6 mice that were intravenously injected with Eμ-Myc lymphoma cells. Mice were fed *ad libitum* with Ctl, Low CHO, and Low PROT diets and were intraperitoneally injected with PBS or αCD8 antibody (Ctl, n = 8; Low CHO, n = 9; Low PROT, n = 10; Low PROT αCD8, n = 6).

(B) Spleen weight of C57BL/6 mice intravenously injected with Eμ-Myc lymphoma cells and fed *ad libitum* with Ctl, Low CHO and Low PROT diets (4 mice per group).

(C and D) Survival curve of NSG mice that were intravenously injected with Eμ-Myc lymphoma cells with or without splenocytes and fed *ad libitum* with Ctl, Low CHO and Low PROT diets (Ctl, n = 5; Low CHO, n = 5; Low PROT, n = 5; Ctl + Splenocytes, n = 3; Low PROT + Splenocytes, n = 3).

(E) Tumor growth curve of syngeneic BALB/c mice that were subcutaneously injected with CT26 colorectal carcinoma cells. Mice were fed *ad libitum* with Ctl and Low PROT (−12.5%, −25%, and −40%) diets (Ctl, n = 7; Low PROT −12,5%, n = 7; Low PROT −25%, n = 8; Low PROT −40%, n = 8).

(F) Tumor volume at day 14 of data shown in (E).

(G) Tumor growth curve of syngeneic BALB/c mice that were subcutaneously injected with CT26 colorectal carcinoma cells. Mice were fed *ad libitum* with Ctl and Low PROT diets and were intraperitoneally injected with PBS or αCD8 antibody (Ctl, n = 11; Low PROT, n = 10; Ctl αCD8, n = 8; Low PROT αCD8, n = 8).

(H) Tumor volume at day 12 of data shown in (G).

*p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent SEM. Comparisons of every group versus Ctl group, except where specified otherwise. When not mentioned, differences are not significant. All experiments are representative of two performed. See also Figure S1.

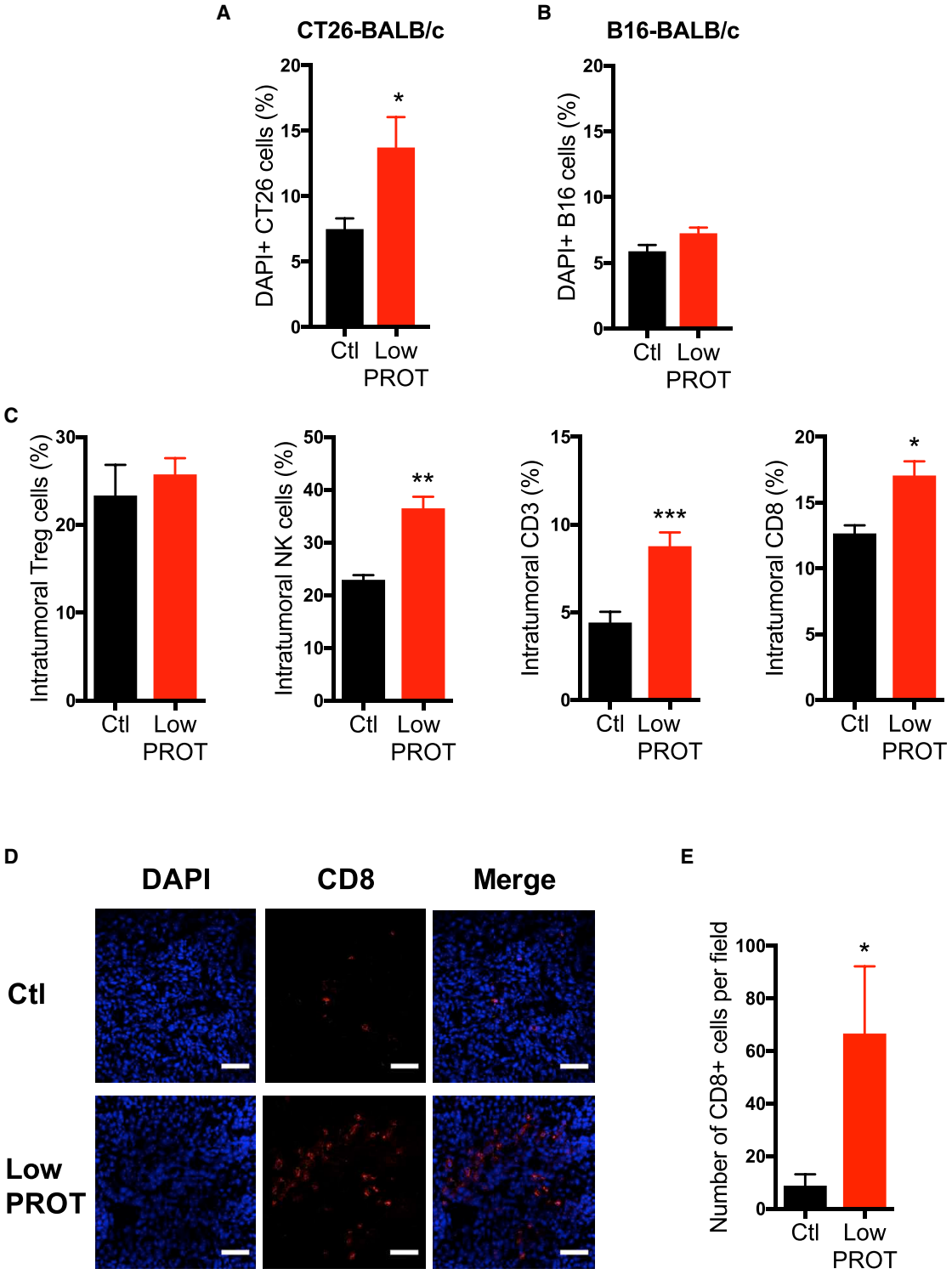


Figure 2. A Low PROT Diet Increases CD8 Tumor-Infiltrating Lymphocytes

(A and B) BALB/c mice were subcutaneously injected with CT26 colorectal carcinoma cells and were fed *ad libitum* with Ctl and Low PROT diets. Upon sacrifice, $CD3^+$ cells were isolated from spleens and incubated with (A) live CT26 cells or (B) B16 cells for 4 hr. The ability of T cells to kill tumor cells was determined by flow cytometry. Cell death of CT26 and B16 cells was determined by DAPI staining (at least seven mice per group).

(legend continued on next page)

immunocompetent BALB/c mice and a murine melanoma cell line (B16) subcutaneously injected into syngeneic immunocompetent C57BL/6 mice. Given that a Low CHO diet did not have any effect on mouse survival using the Eμ-Myc model (Figures 1A and 1B), we focused our attention on the Low PROT diet. We first tested the impact of diet-protein content on tumor growth in the CT26-BALB/c model by generating isocaloric diets presenting a reduction of 12.5%, 25% (as in Figures 1A–1D), or 40% of protein content. The global food intake and mouse weight were equivalent for all diets (Figures S1E and S1F). While −12.5% and −25% protein diets resulted in similar tumor growth reduction, the −40% diet did not show any beneficial effect (Figures 1E and 1F). We therefore decided to use the −25% Low PROT diet (named Low PROT diet) for the rest of the study. Then, using the CT26-BALB/c model (Figures 1G and 1H) and the B16-C57BL/6 model (Figures S1G and S1H), we established that a Low PROT diet attenuated tumor development in an immune-dependent manner, as the depletion of $CD8^+$ T cells (shown in Figure S1I) prevented the beneficial effects mediated by the Low PROT diet (Figures 1G, 1H, S1G, and S1H). Importantly, we verified that food intake or mouse body weight were not affected by the different diets in both models (Figures S1J–S1M). We also verified that glycemia was not affected by the Low PROT diet (Figure S1N).

Altogether, we established using three independent and different mouse cancer models that a Low PROT diet attenuates tumor growth through the induction of an anticancer immune response.

To directly address whether a Low PROT diet can induce an efficient and specific anticancer immune response, we isolated T cells from the spleens of BALB/c mice previously injected with CT26 cells and fed with a control or a Low PROT diet and from C57BL/6 mice previously injected with Eμ-Myc cells and fed a control, Low CHO, or Low PROT diet. We then tested the ability of isolated cytotoxic T cells to kill tumor cells *ex vivo*. In both models, T cells isolated from the spleens of Low PROT-fed mice were more efficient in killing syngeneic tumor cells compared with T cells isolated from control and Low CHO diet-fed mice (Figures 2A, S2A, and S2B). To test whether cytotoxic activity of T cells was specific for the syngeneic tumor cells, we incubated *ex vivo* the T cells isolated from the CT26-BALB/c model with B16 melanoma cells. T cells isolated from the CT26-BALB/c model fed with a Low PROT diet, while efficiently killing CT26 cells (Figure 2A), were unable to kill B16 cells (Figure 2B), thus suggesting that a Low PROT diet leads to the expansion of tumor antigen-specific T cells. Although the percentage of tumor-infiltrating T regulatory cells (Treg, $CD3^+CD4^+CD25^+CD127^-$) did not vary in CT26 tumors, intratumoral natural killer cells (NK, $CD3^-$ $NK1.1^+$) and $CD3^+/CD8^+$ T lymphocytes were increased (Figure 2C). Importantly, this beneficial effect of the Low PROT diet was confirmed and correlated with an increase in $CD8^+$ tumor-infiltrating lymphocytes (TILs) that were visualized by immunofluorescence on tumor sections of BALB/c bearing CT26 tumors (Figures 2D and 2E). The increase in $CD8^+$ TILs was associated with an increase in the mRNA levels of *IFNγ* and its target *CXCL10* in tumors *in vivo* (Figures S2C and S2D).

We demonstrated that a Low PROT diet can limit tumor development not by affecting tumor cell proliferation capacity or by inducing tumor cell death directly but rather through the increase in TILs that results in the induction of an efficient and specific anticancer immune response.

Low-Protein-Diet-Induced Immunosurveillance Requires APCs and Involves INFγ Production by Tumor Cells

Antigen-presenting cells (APCs), like dendritic cells (DCs) or macrophages, are central for antigen presentation (Joffre et al., 2009; Kroemer et al., 2013). We therefore investigated their involvement *in vivo* by depleting phagocytic cells from the myeloid lineage (DCs and macrophages) using liposome clodronate (Van Rooijen and Sanders, 1994) (Figures S3A and S3B). Interestingly, the Low PROT diet-mediated protective effect was lost upon APC depletion (Figures 3A and 3B).

To further support our conclusions, we neutralized CD86, a co-stimulatory protein expressed on APCs that is required for T cell activation, using a blocking antibody *in vivo* (Figure S3C). As for APCs depletion, CD86 blockade *in vivo* prevented the effect of the Low PROT diet on tumor growth (Figures 3C and 3D). Cytokine production by tumor cells, including INFγ, plays a key role in ICD (Galluzzi et al., 2015). We established that reduction of *IFNγ* expression by tumor cells (Figure 3E) prevents the effects of a Low PROT diet on tumor growth (Figure 3F.)

Collectively our data suggest that a Low PROT diet can induce an antitumoral immune response that relies on APCs and, at least in part, on IFNγ production by tumor cells.

A Low-Protein Diet Induces IRE1α-Dependent ER Stress

Since we found that a Low PROT diet induced an antitumoral effect in three independent mouse tumor models, we then sought to investigate the underlying molecular mechanism by monitoring key signaling pathways previously reported as affected by such a regimen. We did not observe significant modulation of the mammalian target of rapamycin (mTOR) pathway or of GCN2 (general control nonderepressible 2) activation, two key sensors of the amino acid content in the cells (Kim et al., 2017; Wek et al., 1995) (Figures S4A–S4C). We also did not observe any modulation of the Akt pathway (Figures S4A and S4B). Moreover, recently, autophagy induction by CR mimetics was associated with an increase in anticancer immunosurveillance (Pietrocola et al., 2016). However, we did not observe any sign of autophagy induction as judged by LC3 conversion in tumor cells isolated from Low PROT-fed mice compared with the control diet (Figures S4D and S4E). Hence, in our model of an isocaloric, mild reduction of protein intake, the increase in immunosurveillance is not mediated by the modulation of macroautophagy or mTOR/GCN2/Akt pathways.

(C) Effect of Ctl and Low PROT diets on the intratumoral frequency of infiltrating Treg, NK, $CD3^+$, and $CD8^+$ cells in CT26 tumors as analyzed by flow cytometry (at least three mice per group).

(D) Immunofluorescent staining of $CD8^+$ T cells in BALB/c CT26 bearing tumors after 25 days of diet. Scale bar is equivalent to 50 μm.

(E) Corresponding quantification (three mice per group).

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Error bars represent SEM. When not mentioned, differences are not significant. See also Figure S2.

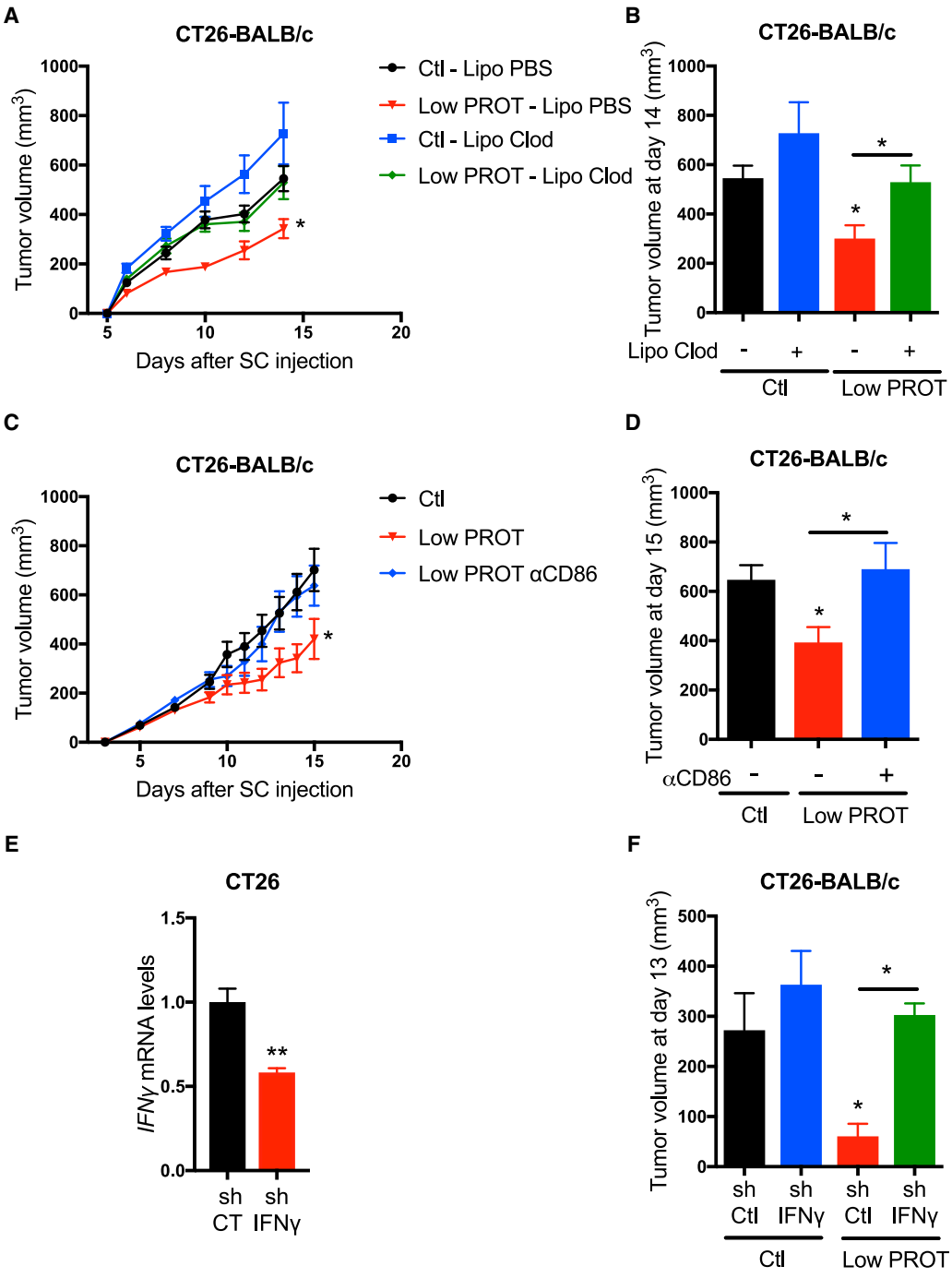


Figure 3. Role of Macrophages and Dendritic Cells on the Antitumor Effect of a Low PROT Diet

BALB/c mice were subcutaneously injected with CT26 colorectal carcinoma cells and were fed *ad libitum* with Ctl and Low PROT diets.

(A) Mice were intraperitoneally injected with clodronate liposomes (Lipo Clod) or PBS liposomes (Lipo PBS) (Ctl-Lipo PBS, n = 8; Low PROT-Lipo PBS, n = 8; Ctl-Lipo Clod, n = 6; Low PROT-Lipo Clod, n = 6).

(B) Tumor volume at day 14 of data shown in (A).

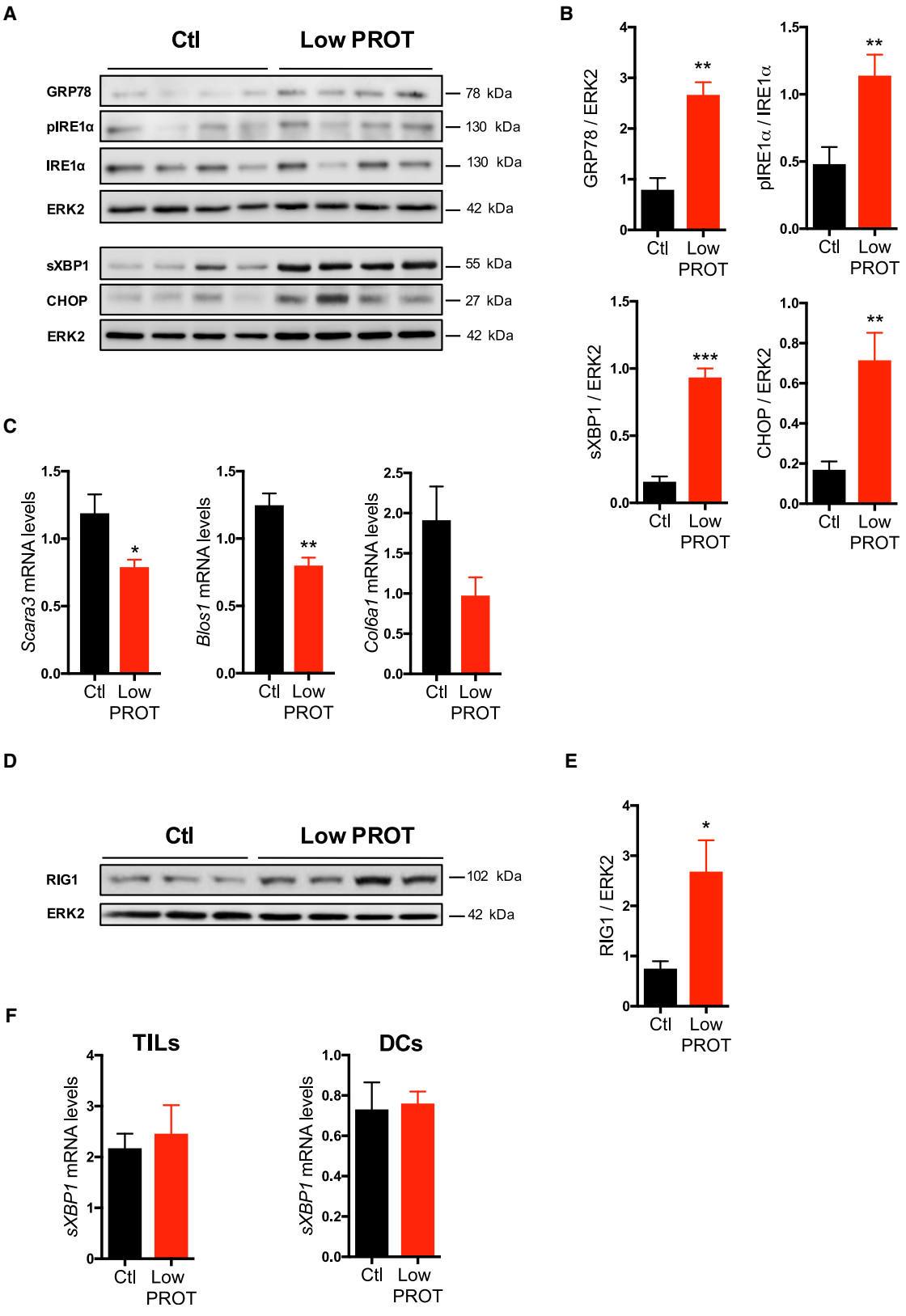
(C) Mice were intraperitoneally injected with PBS or αCD86 antibody (Ctl - PBS, n = 8; Low PROT – PBS, n = 7; Low PROT – αCD86, n = 7).

(D) Tumor volume at day 15 of data shown in (C).

(E) Knockdown in CT26 cells of *IFNγ* using the shRNA technique. *IFNγ* expression was analyzed by qPCR.

(F) BALB/c mice were subcutaneously injected with CT26 shRNA of interferon γ colorectal carcinoma cells and were fed *ad libitum* with Ctl and Low PROT diets. Tumor volume at day 13.

*p < 0.05, **p < 0.01. Error bars represent SEM. Comparisons of every group versus Ctl group, except where specified otherwise. When not mentioned, differences are not significant. See also Figure S3.



(legend on next page)

We then reasoned that reducing protein intake could limit amino acid availability in tumor cells. Knowing that alterations in certain amino acids (such as proline) can control ER stress induction (Jeon et al., 2015; Sahu et al., 2016), we monitored the content of amino acids in tumors. We observed a decrease in most of the amino acids present in tumors isolated from Low PROT diet-fed mice compared with tumors isolated from the control diet group (Figure S4F). We therefore investigated the activation status of different UPR markers in tumors isolated from mice fed with the control or Low PROT diets. GRP78 and CHOP expression were increased and IRE1α phosphorylation was detected in tumor cells isolated from mice fed a Low PROT diet (Figures 4A and 4B), indicating that a Low PROT diet induced the UPR in cancer cells. We did not observe any modulation of the AFT4 and ATF6 branches of the UPR (Figures S5A–S5C).

Given that a Low PROT diet induced the expression of cytokines (*IFNγ* and *CXCL10*) in tumor cells (Figures S1C, S2C, and S2D) and that IRE1α is a central player in this phenomenon (Martinon et al., 2010), we further investigated this pathway. We observed an increase in sXBP1 protein levels in tumor cells obtained from mice fed a Low PROT diet (Figures 4A and 4B). ER stress-dependent IRE1α induction mediated by a Low PROT diet in tumors *in vivo* was further supported by the degradation of RIDD target mRNAs (Figure 4C). RIG1 (retinoic acid inducible gene 1) senses the small RNA fragments generated by RIDD, leading to its activation (Cho et al., 2013). We observed the induction of RIG1 protein levels within the tumors isolated from Low PROT-fed mice (Figures 4D and 4E). Importantly, the activation of IRE1α signaling, as judged by the generation of *sXBP1*, was not observed in TILs nor in DCs, thereby suggesting it is a tumor cell-specific activation (Figure 4F).

Collectively, our data indicate that feeding tumor-bearing mice a Low PROT diet induces an IRE1α-dependent UPR in tumor cells.

IRE1α-Mediated RIG1 Activation in Tumor Cells Is Required for a Low PROT-Induced Anticancer Immune Response

If IRE1α signaling in tumor cells is a central event in the Low PROT-induced anticancer immune response, we reasoned that attenuation of ER stress should prevent the beneficial effect provided by this diet. To test this hypothesis, we first injected CT26 cells in syngeneic immunocompetent BALB/c mice fed *ad libitum* with the control or Low PROT diets. As observed in Figure 1, feeding the mice a Low PROT diet decreased tumor progression compared with mice fed with a control diet (Figures 5A and 5B). Eleven days after subcutaneous injection of tumor cells, half of the mice in each group were treated either with the pan ER stress inhibitor TUDCA or with PBS (vehicle). Strikingly, TUDCA treatment prevented the antitumoral effect of the Low PROT diet, thereby indicating that ER stress is necessary to achieve such response. To further support this observation, we then used the IRE1α-specific RNase inhibitor MKC4485 to block IRE1α-downstream signaling events. Although tumor burden was reduced by the Low PROT diet, this effect was impaired upon treatment with MKC4485 (Figures 5C and 5D). We verified the efficacy of both inhibitors by assessing the reduction in CHOP (for tauroursodeoxycholic acid [TUDCA]) or *sXBP1* expression (for MKC4485) (Figures 5E and 5F) in tumor cells of mice fed with a Low PROT diet. Consistent with our hypothesis, both inhibitors prevented $CD8^+$ T cell infiltration in tumors (Figures 5G, S6A, and S6B), and this effect was associated with a drastic reduction in Low PROT-induced *IFNγ* mRNA levels in tumors (Figure S6C). Importantly, neither the Low PROT diet nor the inhibitors affected T cell populations in lymphoid organs of tumor-bearing mice, suggesting a local modulation of the immune cell infiltrate rather than a global impact on whole immune cell populations (Figures S6D and S6E).

To demonstrate that the Low PROT diet-induced anticancer immune response is controlled by the modulation of IRE1α in cancer cells, we used CT26 cells that were stably silenced for IRE1α (using two independent shRNA sequences, Figure 6A). Importantly, we verified that IRE1α knockdown did not impact on cell proliferation (Figure S7A). We again validated that a Low PROT diet limited tumor growth (Figure 6B). Very importantly, our *in vivo* data also demonstrated that IRE1α knockdown is sufficient to prevent the beneficial effect of a Low PROT diet on tumor growth (Figures 6C, 6D, S7B, and S7C).

To further support our conclusions and to prevent any off-target effects of both small hairpin RNA (shRNA) targeting IRE1α, we invalidated IRE1α in CT26 cells using the CRISPR/Cas9 technology (Figure 6E). Again, IRE1α invalidation did not have an impact on cell proliferation *in vitro* (Figure S7D) but blunted the Low PROT diet-dependent reduction in tumor growth (Figures 6F–6H). Finally, to elucidate the role of RIG1 on the IRE1α-dependent antitumor effect of the Low PROT diet, we invalidated RIG1 in CT26 cells using the CRISPR/Cas9 technique (Figure 6E). As for IRE1α, RIG1 invalidation did not modulate cell proliferation *in vitro* (Figure S7D) but prevented the reduction in tumor growth induced by the Low PROT diet (Figures 6F–6H). We then confirmed that IRE1α invalidation in tumor cells limited the splicing of XBP1 and RIG1 activation induced by the Low PROT diet (Figure 6I), confirming that RIG1 activation is downstream of IRE1α activation.

Figure 4. A Low PROT Diet Causes Unresolved ER Stress in CT26 Tumors

Syngeneic BALB/c mice were subcutaneously injected with CT26 colorectal carcinoma cells and fed *ad libitum* with Ctl and Low PROT diets.

(A) Tumors were harvested after 25 days of diets and lysates were prepared. Expressions of proteins related to the IRE1α pathway were analyzed by western blot.

(B) Average quantification of GRP78, pIRE1α, sXBP1, and CHOP compared with ERK2 levels (used as a loading control) or the corresponding total protein.

(C) mRNA levels of RIDD targets were measured in tumors by qPCR (at least five mice per group).

(D) Expression of RIG1 in tumor tissues was analyzed by western blot.

(E) Average quantification of RIG1 compared with ERK2 levels.

(F) $CD4^+$/$CD8^+$ TILs were isolated from CT26 tumors and DCs were isolated from spleens of BALB/c mice fed with Ctl and Low PROT diets. mRNA levels of *sXBP1* were determined by qPCR and normalized by *uXBP1*.

*p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent SEM. When not mentioned, differences are not significant. See also Figures S4 and S5.

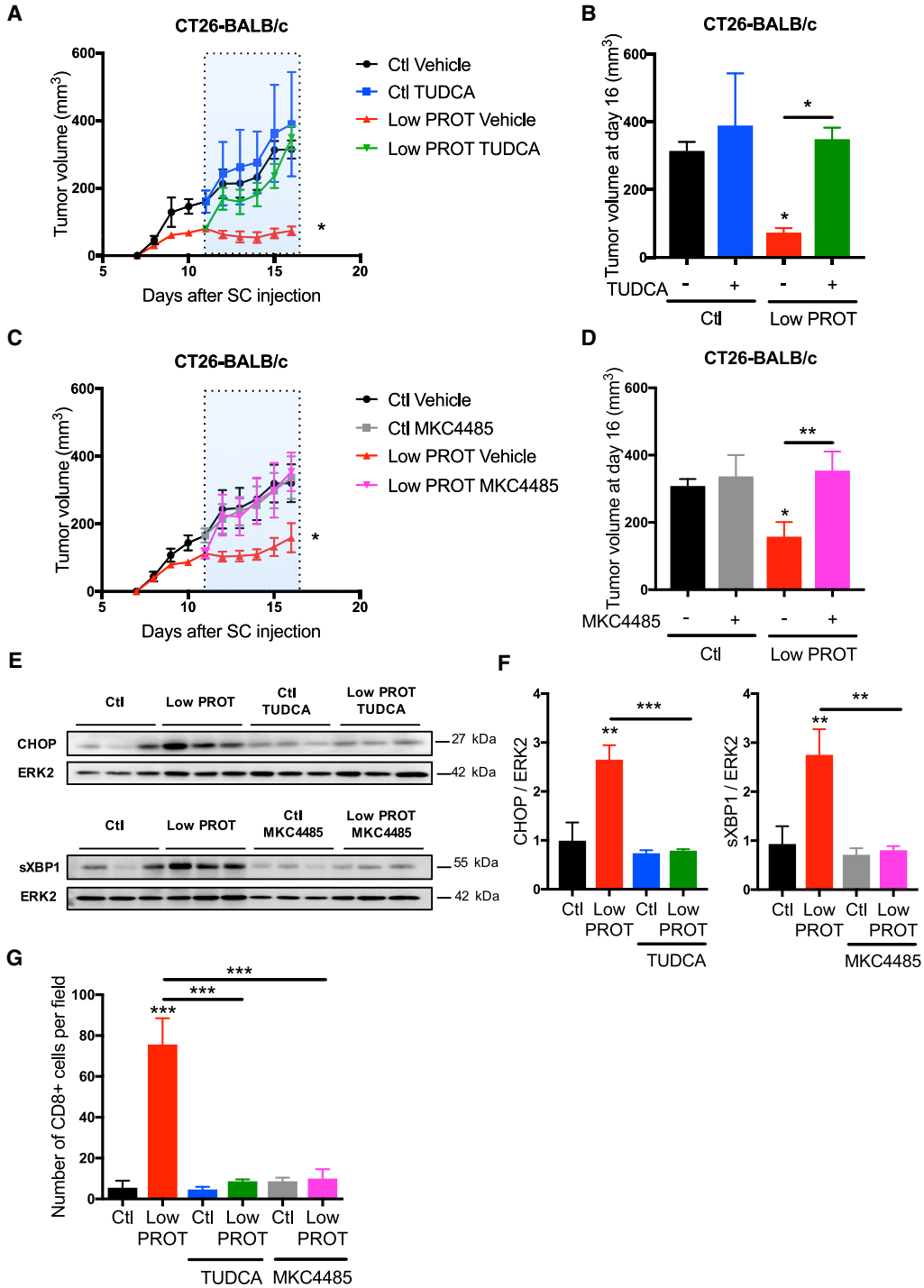


Figure 5. Treatment with ER Stress Inhibitors Reverses the Antitumor Immune Response Induced by a Low PROT Diet

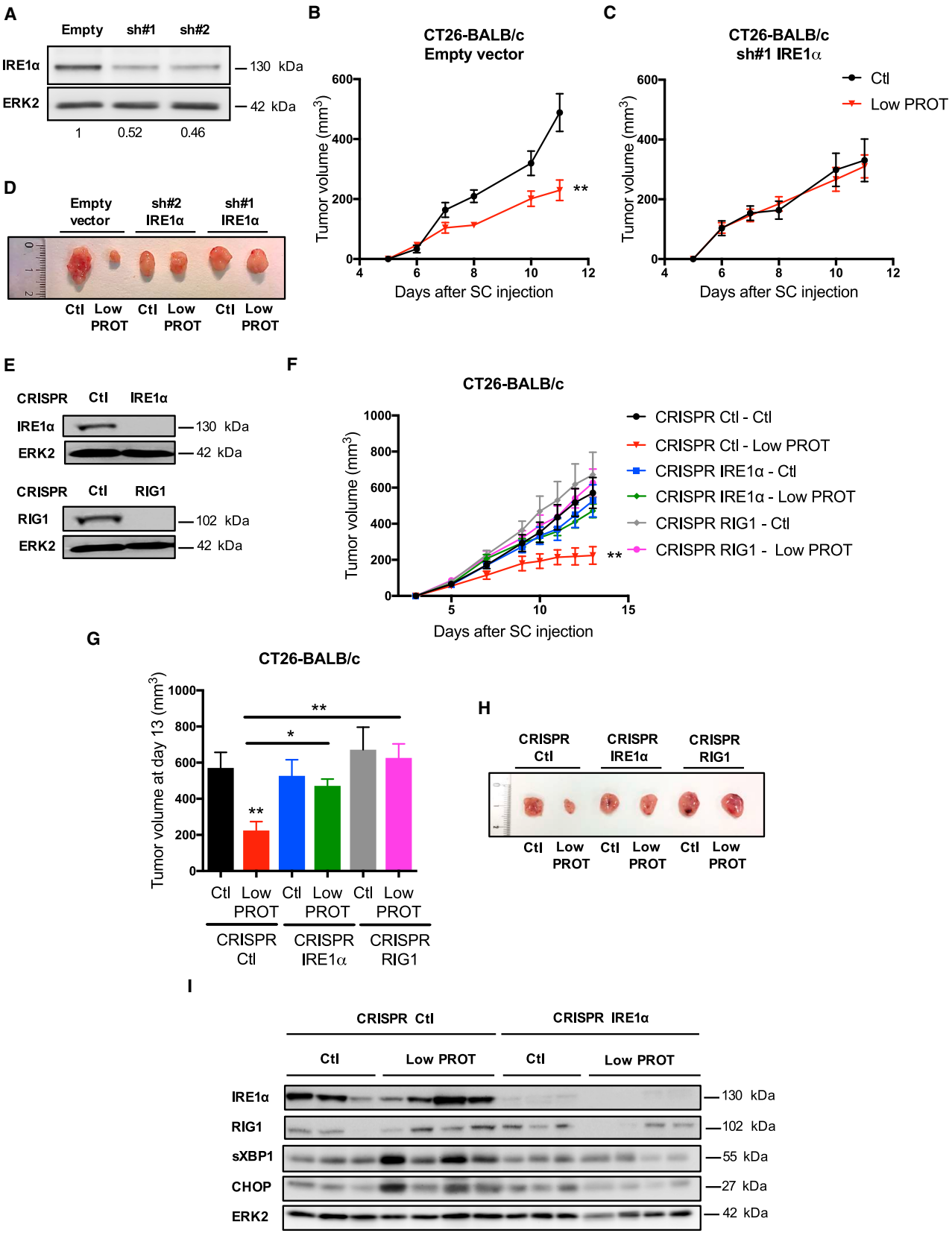
(A–D) Tumor growth curves of syngeneic BALB/c mice that were subcutaneously injected with CT26 colorectal carcinoma cells and were fed *ad libitum* with Ctl and Low PROT diets. Mice were (A) intraperitoneally injected with TUDCA (five mice per group) (C) or administered MKC4485 by gavage from day 11–16 after subcutaneous injection with CT26 cells (at least five mice per group). Average tumor size at day 16 of mice treated with (B) TUDCA or (D) MKC4485 of data shown in (A) and (C).

(E) Tumors were harvested after 30 days of diets and lysates were prepared. Expression of CHOP and sXBP1 were analyzed by western blot.

(F) Corresponding average quantification of CHOP and sXBP1 compared with ERK2 levels (used as a loading control).

(G) Quantification of immunofluorescent staining of $CD8^+$ T cells in tumors shown in Sup Figure 6A (three mice per group).

*p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent SEM. Comparisons of every group versus Ctl group, except where specified otherwise. When not mentioned, differences are not significant. All experiments are representative of two performed. See also Figure S6.



(legend on next page)

Altogether, these results indicate that a Low PROT diet induces IRE1α-mediated RIG1 activation in tumor cells and that this represents a central event in the Low PROT diet-induced immunosurveillance.

Increased IRE1α Signaling Is Associated with Increased Antitumor T Cell Response in Human Cancers

We then decided to corroborate our mouse findings to human data. For that matter, we investigated whether tumor IRE1α activity could be associated with signatures of antitumoral responses. Recently, a gene signature reflecting IRE1α activation was established in glioblastoma (Lhomond et al., 2018). Using TGCA datasets, we stratified glioblastoma (n = 523), melanoma (n = 293), and colorectal cancers (n = 456) based on IRE1α activity and identified for each cancer type IRE1α^{high} and IRE1α^{low} populations (Figures S7E–S7G). Then we investigated the expression of a series of T cell markers (Figures 7A–7C) in both populations in the three cancer types. Interestingly, in both glioblastoma and melanoma, high IRE1α activity correlated with increased levels of T cell markers (Figures 7B–7D). Surprisingly, when the same analysis was carried out in CRC, the enrichment in T cell markers was exclusively observed in grade I tumors (Figures 7A and 7D).

These results suggested that the association between an increase in IRE1α signaling and the increase in T cell recruitment is a common feature observed in human tumors from various origins (Figure 7).

DISCUSSION

In this study, we examined the effect of Low CHO or Low PROT isocaloric diets on tumor growth using three independent immunocompetent syngeneic mouse models: the Eμ-Myc-C57BL/6 B lymphoma model, the B16-C57BL/6 melanoma model, and the CT26-BALB/c CRC model. We established that a 25% reduction in protein but not in carbohydrate intake with no change in calories resulted in a marked decrease in tumor growth. All diets used in our study were isocaloric but had very different impacts on tumor growth (Figure 1). This suggested that the class of nutrients rather than the amount of energy present in the food is essential to limit tumor growth. Very importantly, this effect was not related to the inhibition of cancer cell proliferation or to the induction of cancer cell death *per se* but was rather due to the induction of an efficient antitumoral immune response. Depletion of CD8$^+$ T cells (Figures 1A, 1G, 1H, S1G, and S1H), the use of immunodeficient mice (Figures 1C and 1D) or depletion/blocking of APCs (Figures 3A–3D) prevented the beneficial effect of the Low PROT diet. We also established that feeding tumor-bearing mice with a Low PROT diet induced a tumor-specific cytotoxic T cell response (Figures 2, S2A, and S2B). Therefore, our data challenge the common dogma that lowering protein in the diet limits tumor development by decreasing tumor proliferation. Instead, we demonstrate that a mild reduction in protein intake without a change in total calories ingested induces an adaptive IRE1α-dependent signal in the tumor cells, leading to RIG1 activation. We have also shown that this IRE1α/RIG1-dependent anticancer immune response goes in hand with cytokine production and that this plays a role in the Low PROT diet-induced anticancer immune response as shown for *IFNγ* (Figures 3E and 3F).

Nutrients are main regulators of circulating IGF-1, the levels of which are correlated with increased cancer risk. Consistently, dietary restriction-mediated reduction in IGF-1 is largely described as preventing cancer incidence (Klement and Fink, 2016; Meynet and Ricci, 2014). Restriction of protein intake or some amino acids is more efficient than CR in reducing IGF-1, which consequently inhibits the PI3K/mTOR pathway and therefore limits tumor cell proliferation (Fontana et al., 2008, 2013; Norat et al., 2007; Underwood et al., 1994). However, in our experiments, a mild reduction of dietary protein content (by 25%) slightly, but significantly, reduced the amino acid concentration within tumor tissues but was not associated with a significant modulation of the mTOR pathway (Figures S4A, S4B, and S4F). In addition, we demonstrated that the Low PROT diet-dependent reduction in tumor development was effectively abrogated upon CD8$^+$ T cell or APC depletion (Figures 1A, 1G, 1H, 3A, and 3B) or when using immunodeficient mice (Figures 1C and 1D). Moreover, IRE1α and RIG1 inhibition or invalidation in tumor cells reverted the Low PROT-dependent reduction in cancer development (Figures 5 and 6). Altogether, our results argue against a role of circulating IGF-1 in our settings but indicate that a Low PROT diet induces a tumor cell IRE1α-dependent activation of anticancer-specific T cells in a PI3K/mTOR-independent manner.

The UPR has been described as being either pro- or antitumoral depending on the tumor type, the intensity of the stress, and the nature of the microenvironment (Cubillos-Ruiz et al., 2017; Obacz et al., 2017). We made the observation that mild dietary reduction of protein intake leads to the induction of the UPR in tumor cells (Figure 4). We demonstrated that the beneficial effect of the Low PROT diet is dependent on the endoribonuclease activity of IRE1α (using the MKC4485 RNase inhibitor, Figure 5). Our data therefore suggest that IRE1α activity can

Figure 6. A Low PROT Diet Induces an Anticancer Immune Response in an IRE1α-Dependent Manner

(A) Knockdown in CT26 cells of IRE1α using two different shRNAs. IRE1α expression was analyzed by western blot.

(B and C) (B) Tumor growth curve of BALB/c mice that were subcutaneously injected with CT26 (Empty Vector) colorectal carcinoma cells (C) and with shIRE1α CT26 colorectal carcinoma cells and were fed *ad libitum* with Ctl and Low PROT diets (at least eight mice per group).

(D) Picture of representative dissected tumors for each group.

(E) Invalidation in CT26 cells of IRE1α and RIG1 using the CRISPR/Cas9 technique. IRE1α and RIG1 expression was analyzed by western blot.

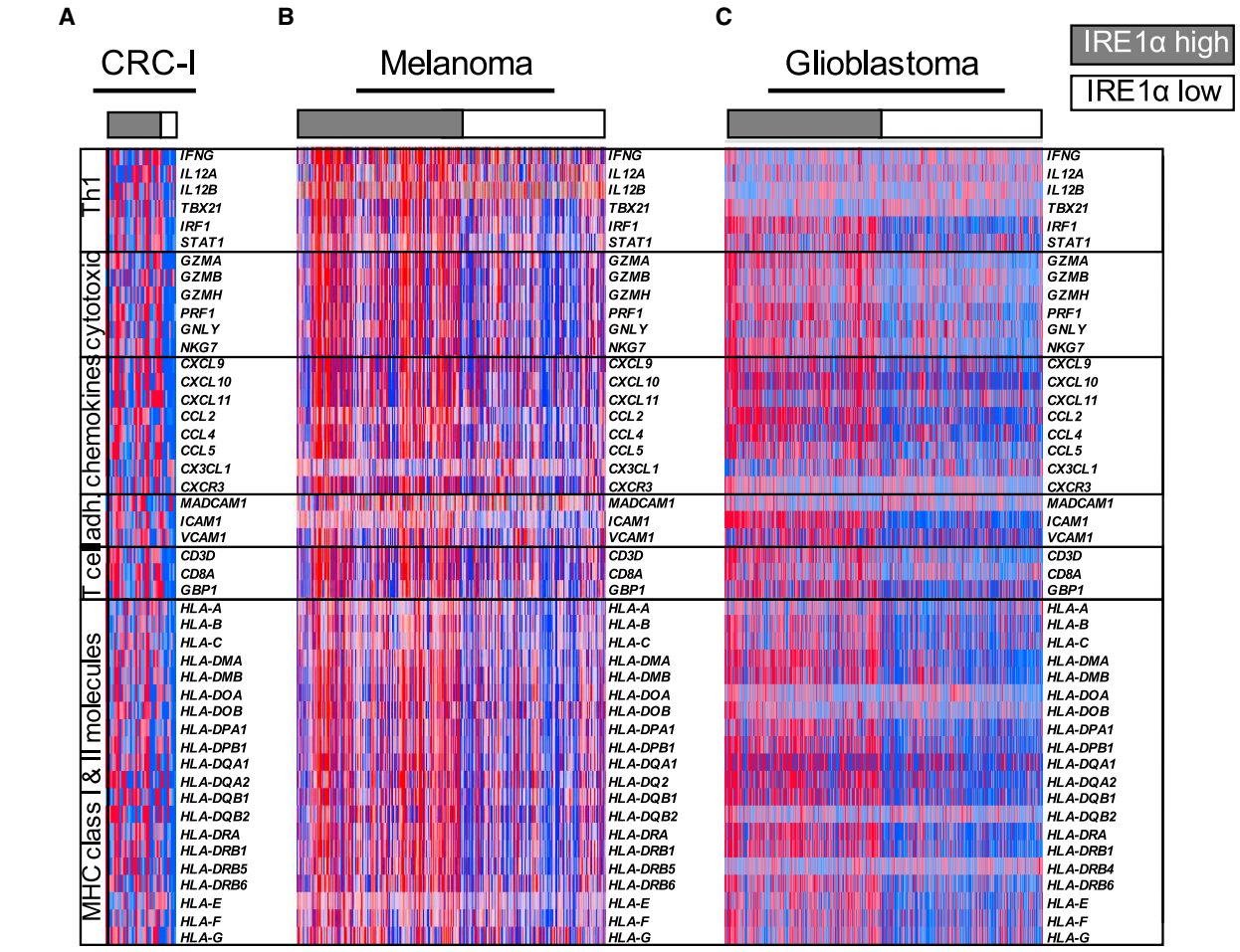
(F) Tumor growth curve of BALB/c mice that were subcutaneously injected with CRISPR Ctl, CRISPR IRE1α and CRISPR RIG1 CT26 cells and were fed *ad libitum* with Ctl and Low PROT diets (at least eight mice per group).

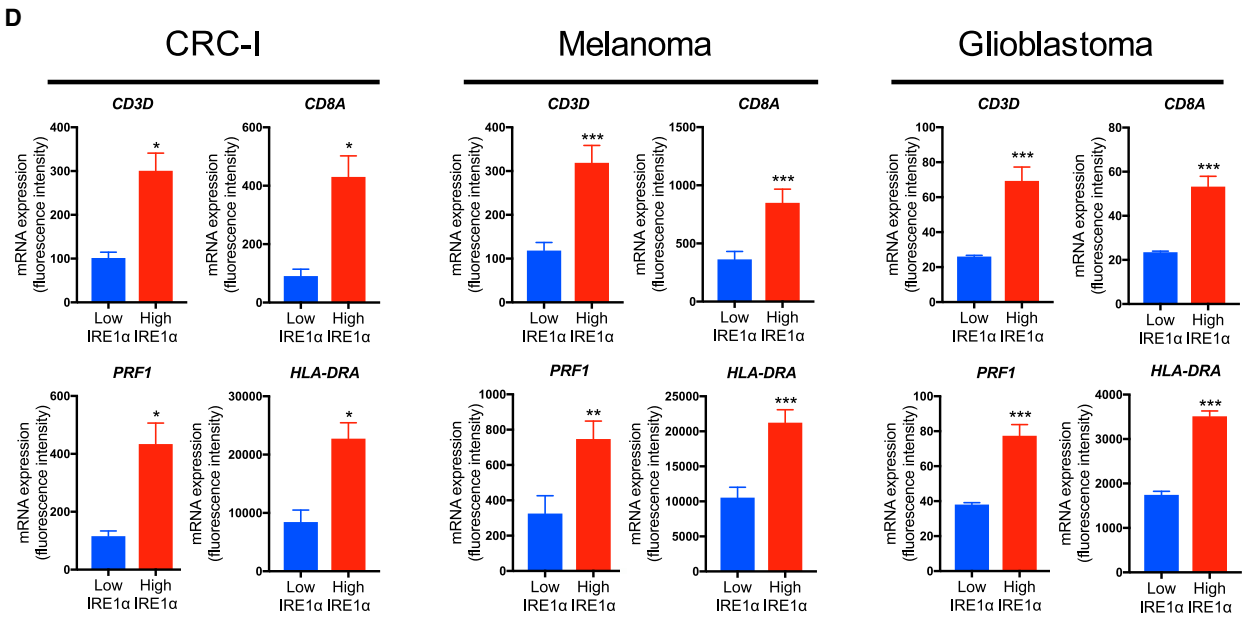
(G) Tumor volume at day 13 of data shown in (F).

(H) Picture of representative dissected tumors for each group.

(I) Tumors were harvested after 17 days of diets and lysates were prepared. Expressions of proteins related to the IRE1α pathway were analyzed by western blot.

*$p < 0.05$, **$p < 0.01$. Error bars represent SEM. Comparisons of every group versus Ctl group, except where specified otherwise. When not mentioned, differences are not significant. See also Figure S7.





(legend on next page)

selectively contribute to the modulation of tumor outcome by impacting on the tumor cells themselves and/or on the nature of the tumor microenvironment.

It was recently established that activation of XBP1 in tumor associated DCs led to a decrease in the anticancer immune response (Cubillos-Ruiz et al., 2015). The authors therefore suggested that targeting ER stress responses could have some relevance in cancer treatment. In our model of Low PROT diet-induced immunosurveillance, we observed the activation of the IRE1α pathway in tumor cells but not in DCs or TILs (Figure 4F). This suggests that this is a tumor cell-specific ER stress response, probably due to the high metabolic demand and sensitivity of tumor cells to ER stress and the UPR. Moreover, this also coincides with tumor IRE1-dependent mechanisms recently described to modulate the nature of the tumor microenvironment (Lhomond et al., 2018). However, our study points toward a careful use of IRE1α inhibitors for cancer treatment.

In the context of our current observations, how IRE1α is activated in tumor cells upon a Low PROT diet remains to be elucidated. One possibility would be that a Low PROT diet, leading to a decrease in specific amino acids in tumors (Figure S4F) results in IRE1α activation, as it was previously suggested (Jeon et al., 2015; Sahu et al., 2016). However, which amino acids are involved remains to be clarified. Another hypothesis could be that somehow a Low PROT diet modulates glycemia that is known to be a central regulator of the UPR (Cubillos-Ruiz et al., 2017). However, we did not observe a difference in glycemia between the two groups (Figure S1N). The exact nature of how IRE1α is activated in tumor cells upon a Low PROT diet remains to be identified, in particular the specific signaling balance between sXBP1 and RIDD. Indeed, it was recently demonstrated that both arms of the IRE1α signaling pathway play antagonistic roles through the remodeling of tumor microenvironment. As such, one might postulate that a Low PROT diet could condition a specific IRE1α signaling code in tumor cells that would favor the recruitment of cytotoxic T cells to the tumor site and prevent the generation of protumoral stroma.

Recently, the use of caloric restriction mimetics (CRm) was shown to induce an anticancer immune response through the induction of autophagy (Pietrocola et al., 2016). However, we did not observe any modulation of autophagy in our settings (Figures S4D and S4E), suggesting that CRm and Low PROT diets utilize independent and possibly complementary pathways to increase immunosurveillance. Here, we describe that a 25% reduction in protein intake is sufficient to induce an efficient anticancer immune response. Of note, this diet did not have a systemic impact on the proportion of immune system populations (Figures S6D and S6E), suggesting a local tumor microenvironment modulation that results in T cell recruitment.

Limitations of Study

Limitation of our work is brought by the fact that there is no established or consensus definition of the percentage of protein reduction that is sufficient to generate a Low PROT diet, a factor that might be individual, tissue, and tumor dependent. Therefore, it remains a challenge to compare studies using strong protein reduction or prolonged fasting with studies using a mild reduction in protein intake. Very low-protein conditions will impact body weight, which may not be suitable for therapeutic approaches (Di Biase et al., 2016). Importantly, in most of these studies, the role of the immune system was not addressed, as experiments were performed in immunodeficient xenograft models. We observed that a drastic reduction in protein content (by 40%) did not lead to a protection toward tumor growth (Figure 1E) in our model, underlying the notion that a mild reduction in protein content may not only be more easily tolerated by patients but also that a massive reduction in protein may prevent the beneficial effect of the diet.

Stimulating the ability of the immune system to fight and limit/eradicate tumor development is among the most promising treatment strategies. The enthusiasm for immunomodulating therapies targeting immune checkpoints results from the success observed in patients suffering from several types of cancer (Callahan et al., 2016). However, this approach is restricted to some types of cancers or some mutations within a cancer type. Here, we describe a simple and efficient method to stimulate the targeted killing of cancer cells by T cells. However, given that mice have a metabolic rate 7-fold higher compared with humans and that they appear to be more resistant to cachexia than humans (Bozzetti and Zupec-Kania, 2016; Demetrius, 2005), the effect of such DR interventions on improvements in immunosurveillance in human cancer prevention should be investigated in future studies. Translating the relevance of our work to humans can be hard to address, as data from cancer patients receiving a Low PROT regimen are not available. For this reason, we investigated the link between IRE1α activation and the increase in antitumor T cells markers (Figure 7). Importantly, regardless of the type of tumor analyzed (CRC, melanoma, or glioblastoma), we observed a strict correlation between a high IRE1α signature and an increase in markers associated with immunosurveillance, suggesting that our results could be relevant to cancer patients.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Mice
  - Cell Lines and Cell Culture Conditions

Figure 7. IRE1α Signaling Signatures and T Cell Markers in CRC, Melanoma, and Glioblastoma

(A–C) mRNA expression of Th1-, cytotoxic mechanisms-, chemokines-, adhesion-, T cell, and MHC class I and II genes based on the groups defined relative to IRE1α activity (High or Low). Probe analysis was carried out from CRC stage I (A), melanoma (B). and glioblastoma (C) tumors from patients according to tumor IRE1α status (i.e., IRE1α high [gray boxes] and IRE1α low [white boxes] groups).

(D) Significant representative genes modulated in IRE1α low versus high tumors. *PRF1*: Perforin-1, *HLA-DRA*: HLA class II histocompatibility antigen, DR alpha chain, *CD3D*: T cell surface glycoprotein CD3 delta chain, *CD8A*: T cell surface glycoprotein CD8 alpha chain.

*p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent SEM. See also Figure S7.

- METHOD DETAILS
  - Cytotoxicity Assay
  - Flow Cytometry Analysis
  - Confocal Laser Scanning Microscopy
  - Western Blot Analysis
  - Reverse Transcriptase Quantitative-PCR (RT-qPCR) Analysis
  - Generation of shIFNγ- and shIRE1α-Transduced Cells
  - Generation of CRISPR/Cas9 Cells
  - Sampling of Intracellular Metabolites
  - Analysis of Amino Acid Residues by Liquid Chromatography Coupled to High Resolution Mass Spectrometry (LC-MS)
  - Bioinformatic Analysis
  - Statistics

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and can be found with this article online at https://doi.org/10.1016/j.cmet.2018.02.009.

ACKNOWLEDGMENTS

We gratefully acknowledge the Centre Méditerranéen de Médecine Moléculaire (C3M) animal and confocal facilities. We thank Maeva Gesson and Drs. Claudio Hetz, Bruno Segui, Frederic Bost and Cédric Chaveroux, Sophie Tartare-Deckert, Benjamin Salucki, Frederic Larbret, and Caetano Reis e Sousa for reagents and help. This work was supported by the Fondation ARC pour la Recherche sur le Cancer, the Agence Nationale de la Recherche (LABEX SIGNALIFE ANR-11-LABX-0028-01) and Inserm. This study was partly supported by research funding from the Canceropôle PACA, Institut National du Cancer and Conseil Régional PACA, by CEA and the French Ministry of Research and National Research Agency as part of the French metabolomics and fluxomics infrastructure (MetaboHUB, ANR-11-INBS-0010 grant). L.M. was supported by la Fondation pour la Recherche Médicale (FRM) and E. Villa by La Ligue contre le Cancer. E.C. is funded by grants from Institut National Du Cancer (INCa PLBIO: 2015-111 and INCA-7981), Ligue Contre le Cancer (Comité des Landes, LARGE project), and EU H2020 MSCA ITN-675448 (TRAINERS). B.B.-M. was supported by grants from Société Francophone du Diabète and Association Française pour l'Etude du Foie (AFEF)/Aptalis and by the Fondation ARC.

AUTHOR CONTRIBUTIONS

C.R.-P. and J.P.B performed the majority of research described herein and were assisted by G.M.D.D, L.M., E. Villa, L.E.A., J.C., R.M., and E. Verhoeyen, S.M., C.L., B.B.-M., E.C., and T.P. provided intellectual input and experiments. J.B.P. and E.C. provided invaluable reagents. K.V., A.C., T.A., and E.C. performed bioinformatic analysis of the IRE1α gene signature. F.A.C., P.L., E.C.-V., and F.F. performed amino acid measurements. J.-E.R. designed research, secured funding, and wrote the manuscript.

DECLARATION OF INTERESTS

E.C. is a founding member of Cell Stress Discoveries. J.B.P. is an employee of and owns stock in Fosun Orinove. The IRE1 signature is patented: EP17306855.2. Remaining authors declare no competing interests.

Received: June 26, 2017
Revised: December 19, 2017
Accepted: February 12, 2018
Published: March 15, 2018

REFERENCES

Adams, J.M., Harris, A.W., Pinkert, C.A., Corcoran, L.M., Alexander, W.S., Cory, S., Palmiter, R.D., and Brinster, R.L. (1985). The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. Nature *318*, 533–538.

Bénéteau, M., Zunino, B., Jacquin, M.A., Meynet, O., Chiche, J., Pradelli, L.A., Marchetti, S., Cornille, A., Carles, M., and Ricci, J.E. (2012). Combination of glycolysis inhibition with chemotherapy results in an antitumor immune response. Proc. Natl. Acad. Sci. USA *109*, 20071–20076.

Bozzetti, F., and Zupec-Kania, B. (2016). Toward a cancer-specific diet. Clin. Nutr. *35*, 1188–1195.

Calfon, M., Zeng, H., Urano, F., Till, J.H., Hubbard, S.R., Harding, H.P., Clark, S.G., and Ron, D. (2002). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature *415*, 92–96.

Callahan, M.K., Postow, M.A., and Wolchok, J.D. (2016). Targeting T cell co-receptors for cancer therapy. Immunity *44*, 1069–1078.

Cho, J.A., Lee, A.H., Platzer, B., Cross, B.C., Gardner, B.M., De Luca, H., Luong, P., Harding, H.P., Glimcher, L.H., Walter, P., et al. (2013). The unfolded protein response element IRE1alpha senses bacterial proteins invading the ER to activate RIG-I and innate immune signaling. Cell Host Microbe *13*, 558–569.

Cubillos-Ruiz, J.R., Bettigole, S.E., and Glimcher, L.H. (2017). Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. Cell *168*, 692–706.

Cubillos-Ruiz, J.R., Silberman, P.C., Rutkowski, M.R., Chopra, S., Perales-Puchalt, A., Song, M., Zhang, S., Bettigole, S.E., Gupta, D., Holcomb, K., et al. (2015). ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. Cell *161*, 1527–1538.

Demetrius, L. (2005). Of mice and men. When it comes to studying ageing and the means to slow it down, mice are not just small humans. EMBO Rep. *6* (Spec No), S39–S44.

Di Biase, S., Lee, C., Brandhorst, S., Manes, B., Buono, R., Cheng, C.W., Cacciottolo, M., Martin-Montalvo, A., de Cabo, R., Wei, M., et al. (2016). Fasting-mimicking diet reduces HO-1 to promote T cell-mediated tumor cytotoxicity. Cancer Cell *30*, 136–146.

Eckard, S.C., Rice, G.I., Fabre, A., Badens, C., Gray, E.E., Hartley, J.L., Crow, Y.J., and Stetson, D.B. (2014). The SKIV2L RNA exosome limits activation of the RIG-I-like receptors. Nat. Immunol. *15*, 839–845.

Fontana, L., Adelaiye, R.M., Rastelli, A.L., Miles, K.M., Ciamporcero, E., Longo, V.D., Nguyen, H., Vessella, R., and Pili, R. (2013). Dietary protein restriction inhibits tumor growth in human xenograft models. Oncotarget *4*, 2451–2461.

Fontana, L., and Partridge, L. (2015). Promoting health and longevity through diet: from model organisms to humans. Cell *161*, 106–118.

Fontana, L., Weiss, E.P., Villareal, D.T., Klein, S., and Holloszy, J.O. (2008). Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans. Aging Cell *7*, 681–687.

Frecha, C., Fusil, F., Cosset, F.L., and Verhoeyen, E. (2011). In vivo gene delivery into hCD34+ cells in a humanized mouse model. Methods Mol. Biol. *737*, 367–390.

Galluzzi, L., Buque, A., Kepp, O., Zitvogel, L., and Kroemer, G. (2015). Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell *28*, 690–714.

Garg, A.D., Galluzzi, L., Apetoh, L., Baert, T., Birge, R.B., Bravo-San Pedro, J.M., Breckpot, K., Brough, D., Chaurio, R., Cirone, M., et al. (2015). Molecular and translational classifications of DAMPs in immunogenic cell death. Front. Immunol. *6*, 588.

Hetz, C., Chevet, E., and Oakes, S.A. (2015). Proteostasis control by the unfolded protein response. Nat. Cell Biol. *17*, 829–838.

Jeon, Y.J., Khelifa, S., Ratnikov, B., Scott, D.A., Feng, Y., Parisi, F., Ruller, C., Lau, E., Kim, H., Brill, L.M., et al. (2015). Regulation of glutamine Carrier proteins by RNF5 determines breast cancer response to ER stress-inducing chemotherapies. Cancer Cell *27*, 354–369.

Joffre, O., Nolte, M.A., Spörri, R., and Reis e Sousa, C. (2009). Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. Immunol. Rev. *227*, 234–247.

Kim, L.C., Cook, R.S., and Chen, J. (2017). mTORC1 and mTORC2 in cancer and the tumor microenvironment. Oncogene *36*, 2191–2201.

Klement, R.J., and Fink, M.K. (2016). Dietary and pharmacological modification of the insulin/IGF-1 system: exploiting the full repertoire against cancer. Oncogenesis *5*, e193.

Kroemer, G., Galluzzi, L., Kepp, O., and Zitvogel, L. (2013). Immunogenic cell death in cancer therapy. Annu. Rev. Immunol. *31*, 51–72.

Lee, K., Tirasophon, W., Shen, X., Michalak, M., Prywes, R., Okada, T., Yoshida, H., Mori, K., and Kaufman, R.J. (2002). IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. Genes Dev. *16*, 452–466.

Levine, M.E., Suarez, J.A., Brandhorst, S., Balasubramanian, P., Cheng, C.W., Madia, F., Fontana, L., Mirisola, M.G., Guevara-Aguirre, J., Wan, J., et al. (2014). Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. Cell Metab. *19*, 407–417.

Lhomond, S., Avril, T., Dejeans, N., Voutetakis, K., Doultsinos, D., McMahon, M., Pineau, R., Obacz, J., Papadodima, O., Jouan, F., et al. (2018). Dual IRE1 RNase functions dictate glioblastoma development. EMBO Mol. Med., in press, pii: e7929. https://doi.org/10.15252/emmm.201707929.

Longo, V.D., and Mattson, M.P. (2014). Fasting: molecular mechanisms and clinical applications. Cell Metab. *19*, 181–192.

Lu, Y., Liang, F.X., and Wang, X. (2014). A synthetic biology approach identifies the mammalian UPR RNA ligase RtcB. Mol. Cell *55*, 758–770.

Lu, Z., Xie, J., Wu, G., Shen, J., Collins, R., Chen, W., Kang, X., Luo, M., Zou, Y., Huang, L.J., et al. (2017). Fasting selectively blocks development of acute lymphoblastic leukemia via leptin-receptor upregulation. Nat. Med. *23*, 79–90.

Martinon, F., Chen, X., Lee, A.H., and Glimcher, L.H. (2010). TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. Nat. Immunol. *11*, 411–418.

Maurel, M., Chevet, E., Tavernier, J., and Gerlo, S. (2014). Getting RIDD of RNA: IRE1 in cell fate regulation. Trends Biochem. Sci. *39*, 245–254.

Meynet, O., and Ricci, J.E. (2014). Caloric restriction and cancer: molecular mechanisms and clinical implications. Trends Mol. Med. *20*, 419–427.

Meynet, O., Zunino, B., Happo, L., Pradelli, L.A., Chiche, J., Jacquin, M.A., Mondragon, L., Tanti, J.F., Taillan, B., Garnier, G., et al. (2013). Caloric restriction modulates Mcl-1 expression and sensitizes lymphomas to BH3 mimetic in mice. Blood *122*, 2402–2411.

Norat, T., Dossus, L., Rinaldi, S., Overvad, K., Grønbaek, H., Tjønneland, A., Olsen, A., Clavel-Chapelon, F., Boutron-Ruault, M.C., Boeing, H., et al. (2007). Diet, serum insulin-like growth factor-I and IGF-binding protein-3 in European women. Eur. J. Clin. Nutr. *61*, 91–98.

Obacz, J., Avril, T., Rubio-Patiño, C., Bossowski, J.P., Igbaria, A., Ricci, J.E., and Chevet, E. (2017). Regulation of tumor-stroma interactions by the unfolded protein response. FEBS J. https://doi.org/10.1111/febs.14359.

Pakos-Zebrucka, K., Koryga, I., Mnich, K., Ljujic, M., Samali, A., and Gorman, A.M. (2016). The integrated stress response. EMBO Rep. *17*, 1374–1395.

Pietrocola, F., Pol, J., Vacchelli, E., Rao, S., Enot, D.P., Baracco, E.E., Levesque, S., Castoldi, F., Jacquelot, N., Yamazaki, T., et al. (2016). Caloric restriction mimetics enhance anticancer immunosurveillance. Cancer Cell *30*, 147–160.

Pluquet, O., Dejeans, N., Bouchecareilh, M., Lhomond, S., Pineau, R., Higa, A., Delugin, M., Combe, C., Loriot, S., Cubel, G., et al. (2013). Posttranscriptional regulation of PER1 underlies the oncogenic function of IREα. Cancer Res. *73*, 4732–4743.

Porporato, P.E. (2016). Understanding cachexia as a cancer metabolism syndrome. Oncogenesis *5*, e200.

Rubio-Patiño, C., Bossowski, J.P., Villa, E., Mondragon, L., Zunino, B., Proics, E., Chiche, J., Bost, F., Verhoeyen, E., and Ricci, J.E. (2016). Low carbohydrate diet prevents Mcl-1-mediated resistance to BH3-mimetics. Oncotarget *7*, 73270–73279.

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., and Smyth, G.K. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. *43*, e47.

Sahu, N., Dela Cruz, D., Gao, M., Sandoval, W., Haverty, P.M., Liu, J., Stephan, J.P., Haley, B., Classon, M., Hatzivassiliou, G., et al. (2016). Proline starvation induces unresolved ER stress and hinders mTORC1-dependent tumorigenesis. Cell Metab. *24*, 753–761.

Solon-Biet, S.M., McMahon, A.C., Ballard, J.W., Ruohonen, K., Wu, L.E., Cogger, V.C., Warren, A., Huang, X., Pichaud, N., Melvin, R.G., et al. (2014). The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. Cell Metab. *19*, 418–430.

Solon-Biet, S.M., Mitchell, S.J., Coogan, S.C., Cogger, V.C., Gokarn, R., McMahon, A.C., Raubenheimer, D., de Cabo, R., Simpson, S.J., and Le Couteur, D.G. (2015). Dietary protein to carbohydrate ratio and caloric restriction: comparing metabolic outcomes in mice. Cell Rep. *11*, 1529–1534.

Underwood, L.E., Thissen, J.P., Lemozy, S., Ketelslegers, J.M., and Clemmons, D.R. (1994). Hormonal and nutritional regulation of IGF-I and its binding proteins. Horm. Res. *42*, 145–151.

Van Rooijen, N., and Sanders, A. (1994). Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. J. Immunol. Methods *174*, 83–93.

Villeneuve, J., Lepreux, S., Mulot, A., Bérard, A.M., Higa-Nishiyama, A., Costet, P., De Ledinghen, V., Bioulac-Sage, P., Balabaud, C., Nurden, A.T., et al. (2010). A protective role for CD154 in hepatic steatosis in mice. Hepatology *52*, 1968–1979.

Wek, S.A., Zhu, S., and Wek, R.C. (1995). The histidyl-tRNA synthetase-related sequence in the eIF-2 alpha protein kinase GCN2 interacts with tRNA and is required for activation in response to starvation for different amino acids. Mol. Cell. Biol. *15*, 4497–4506.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
FITC Anti-CD19	Miltenyi Biotec	130-102-494; RRID:AB_2661108
FITC Anti-CD45R	Miltenyi Biotec	130-110-845; RRID:AB_2658273
FITC Anti-CD49B	BD Biosciences	553857; RRID:AB_395093
FITC Anti-CD11b	BD Biosciences	553310; RRID:AB_394774
FITC Anti-TER-119	BD Biosciences	557915; RRID:AB_396936
VioBlue Anti-CD4	BD Biosciences	558107; RRID:AB_397030
PE Anti-CD8	BD Biosciences	553032; RRID:AB_394570
PE Anti NK1.1	BD Biosciences	557391; RRID:AB_396674
PE Anti-CD11c	BD Biosciences	557401; RRID:AB_396684
PECy7 Anti-CD86	BD Biosciences	560582; RRID:AB_1727518
APC Anti-CD127	BD Biosciences	564175
FITC Anti-CD3	eBioscience	11-0031-85; RRID:AB_464883
VioBlue Anti-F4/80	eBioscience	48-4801-82; RRID:AB_1548747
APCCy7 Anti-CD25	BD Biosciences	557658; RRID:AB_396773
Mouse monoclonal Anti-XBP1	Santa Cruz	sc-8015; RRID:AB_628449
Mouse monoclonal Anti-ERK2	Santa Cruz	sc-1647; RRID:AB_627547
Rabbit monoclonal Anti-IRE1α	Cell Signaling	3294; RRID:AB_823545
Rabbit monoclonal Anti-pS6K	Cell Signaling	9234; RRID:AB_2269803
Rabbit polyclonal Anti-S6K	Cell Signaling	9202; RRID:AB_331676
Rabbit polyclonal Anti-pAKT	Cell Signaling	9271; RRID:AB_329825
Mouse monoclonal Anti-AKT	Cell Signaling	2967; RRID:AB_331160
Rabbit monoclonal Anti-LC3B	Cell Signaling	3868; RRID:AB_2137707
Rabbit polyclonal Anti-eIF2α	Cell Signaling	9722; RRID:AB_2230924
Rabbit polyclonal Anti-peIF2α	Cell Signaling	9721; RRID:AB_330951
Rabbit monoclonal Anti-RIG1	Cell Signaling	3743; RRID:AB_2269233
Rabbit monoclonal Anti-ATF4	Cell Signaling	11815; RRID:AB_2616025
Mouse monoclonal Anti-CHOP	Cell Signaling	2895; RRID:AB_2089254
Rabbit polyclonal Anti-GCN2	Cell Signaling	3302; RRID:AB_2277617
Mouse monoclonal Anti-ATF6	Novus biologicals	NBP1-40256; RRID:AB_2058774
Rabbit polyclonal Anti-pIREα	Novus biologicals	NB100-2323; RRID:AB_10145203
Rabbit polyclonal Anti-GRP78	Abcam	ab21685; RRID:AB_2119834
Rabbit monoclonal Anti-pGCN2	Abcam	ab75836; RRID:AB_1310260
anti-CD8-depleting antibody (clone53-6.7)	Bioxcell	BE0004-1; RRID:AB_1107671
anti-CD86-blocking antibody (clone GL-1)	Bioxcell	BE0025; RRID:AB_1107678
Purified Anti-mouse CD8a (clone 53-6.7)	Biolegend	100701; RRID:AB_312740
Alexa Fluor 594 anti-rat secondary antibody	Molecular Probes	A11007; RRID:AB_141374
Chemicals, Peptides, and Recombinant Proteins		
jetPEI DNA transfection Reagent	PolyPlus Transfection	POL101-10N
Clodronate Liposomes and PBS Liposomes	Liposoma B.V.	PBS-02
TUDCA	Sigma-Aldrich	T0266
MKC4485	John B. Patterson	N/A
Recombinant mouse IL-2	AbD Serotech	PMP38
DAPI	Sigma-Aldrich	D9542
Fast Sybr Green	Applied Biosystems	4385616

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Taqman Fast Universal PCR Master Mix	Applied Biosystems	4352042
O.C.T. compound	Tissue-Tek	4583
amino acids mixture (98 atom % 13C, 98 atom % 15N)	Sigma-Aldrich	608254
Critical Commercial Assays		
Mouse Tumor Dissociation Kit	Miltenyi Biotec	130-096-730
Mouse CD4/CD8 (TIL) Microbeads	Miltenyi Biotec	130-116-480
Mouse CD11c Microbeads ultrapure	Miltenyi Biotec	130-108-338
ECL Western Blotting Detection Reagents	GE Healthcare	RPN2106
Pierce BCA Protein Assay Kit	Thermo Fisher Scientific	23225
RNAeasy minikit	Qiagen	74104
Omniscript RT kit	Qiagen	205113
Experimental Models: Cell Lines		
Mouse B16-F1 cells	ATCC	CRL-6323
Mouse CT26.WT cells	ATCC	CRL-2638
Human Embrionic Kidney-293T Cells	ATCC	CRL-1573
Experimental Models: Organisms/Strains		
Mouse: Eμ-Myc	The Jackson Laboratory	002728
Mouse: C57BL/6JOlaHsd	ENVIGO	N/A
Mouse: BALB/cOlaHsd	ENVIGO	N/A
Mouse: NOD scid gamma $c^{-/-}$ (NSG)	The Jackson Laboratory	005557
Oligonucleotides		
Atf3 (Mm00476033_m1)	Applied Biosystems	4331182
Ero1lb (Mm00470754_m1)	Applied Biosystems	4331182
Sars (Mm00803379_m1)	Applied Biosystems	4331182
Trib3 (Mm00454879_m1)	Applied Biosystems	4331182
Hsp90b1 (Mm00441926_m1)	Applied Biosystems	4331182
Hyou1 (Mm00491279_m1)	Applied Biosystems	4331182
Rn18s (Mm03928990_g1)	Applied Biosystems	4331182
Scara3 Forward TGCATGGATACTGACCCTGA	This paper	N/A
Scara3 Reverse GCCGTGTTACCAGCTTCTTC	This paper	N/A
Blos1 Forward CAAGGAGCTGCAGGAGAAGA	This paper	N/A
Blos1 Reverse GCCTGGTTGAAGTTCTCCAC	This paper	N/A
Col6 Forward TGCTCAACATGAAGCAGACC	This paper	N/A
Col6 Reverse TTGAGGGAGAAAGCTCTGGA	This paper	N/A
IFNγ Forward TCAAGTGGCATAGATGTGGAAGAA	This paper	N/A
IFNγ Reverse TGGCTCTGCAGGATTTTCATG	This paper	N/A
CXCL10 Forward GCTGATGCAGGTACAGCGT	This paper	N/A
CXCL10 Reverse 5′-CACCATGAATCAAACTGCGA	This paper	N/A
βactin Forward TGGAATCCTGTGGCATCCATGAAA	This paper	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
βactin Reverse TAAAACGCAGCTCAGTAACAGTCCG	This paper	N/A
sXBP1 Forward GCTGAGTCCGCAGCAGGTG	Villeneuve et al., 2010	N/A
uXBP1 Forward GAGTCCGCAGCACTCAGACT	Villeneuve et al., 2010	N/A
XBP1 Reverse GTGTCAGAGTCCATGGGAAGA	Villeneuve et al., 2010	N/A
shRNA target sequence: IRE1α sh#1: CCAAGATGCTGGAGAGATT	This paper	N/A
shRNA target sequence: IRE1α sh#2: GCTCGTGAATTGATAGAGA	This paper	N/A
shControl	Santa Cruz	sc-108060
shIFNγ	Santa Cruz	sc-39607-SH
Recombinant DNA		
psPAX2 plasmid	Addgene	12260
pSUPER	Oligoengine	VEC-PRT-0005/0006
MLV-Gag-Pol	Els Verhoeyen	N/A
CRISPR–Cas9-control plasmid	Santa Cruz	sc-418922
CRISPR–Cas9-IRE1α plasmid	Santa Cruz	sc-429758
CRISPR–Cas9-RIG1 plasmid	Santa Cruz	sc-432915
Software and Algorithms		
Bioinfominer	e-NIOS	www.bioinfominer.com
GraphPad Prism 7	GraphPad software	https://www.graphpad.com/scientific-software/prism/
Image J	NIH	https://imagej.nih.gov/ij/
Xcalibur 2.1	Thermo Fisher Scientific	N/A
Other		
Control Diet	ENVIGO	TD.130931
Low CHO Diet	ENVIGO	TD.130932
Low PROT Diet -12,5%	ENVIGO	TD.170630
Low PROT Diet -25%	ENVIGO	TD.130933
Low PROT Diet -40%	ENVIGO	TD.170631
Dulbecco's MEM (DMEM) w/o Amino Acids	USBiological	D9800-13

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jean-Ehrland Ricci (ricci@unice.fr).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

All animal experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee and the regional ethics committee (approval reference PEA-232 and PEA-233). All experiments used age-matched female littermates. Eμ-Myc/wild-type (WT) mice were obtained from The Jackson Laboratory (#002728). Five-week-old WT syngeneic C57BL/6 mice and BALB/c mice were obtained from ENVIGO. NOD scid gamma $c^{-/-}$ (NSG) mice were obtained from The Jackson Laboratory (#005557) and housed in our animal facilities (C3M-Nice, France).

Mice were fed with isocaloric diets generated by ENVIGO: Control (Ctl: TD.130931), low carbohydrates (Low CHO: TD.130932) and low protein diet (Low PROT or Low PROT -25%: TD.130933). When specified, two other low protein diets were used (Low

PROT -12.5%: TD.170630 and Low PROT -40%: TD.170631). % energy CHO:PROT:FAT: Ctl - (70.9%:19.5%:9.6%); Low CHO - (54%:26.9%:19.2%); Low PROT -25% - (73.7%:14.9%:11.5%); Low PROT -12.5% - (72.2%:17%:10.8%); Low PROT -40% - (76.4%:12.2%:11.4%).

WT syngeneic C57BL/6 mice were intravenously injected with 0.1 × 10^6 Eμ-Myc cells. At day four after injection, the food was replaced with isocaloric diets generated by ENVIGO for 2 weeks: Ctl, Low CHO or Low PROT. BALB/c and C57BL/6 mice were fed with isocaloric Ctl and Low PROT diets (-12.5%, -25%, -40%) one week before subcutaneous injection with 0.5 x 10^6 CT26 cells or 0.25 x 10^6 B16 cells. NOD scid gamma $c^{-/-}$ (NSG) mice were fed with Ctl, Low CHO or Low PROT isocaloric diets generated by ENVIGO one week before intravenous injection with 0.1×10^6 Eμ-Myc cells. For the groups with splenocyte co-injection, splenocytes from wild-type C57BL/6 mice were freshly isolated and washed in PBS. Then, 2×10^6 splenocytes were co-injected with 0.1×10^6 Eμ-Myc cells.

Syngeneic C57BL/6 mice and NSG mice injected with Eμ-Myc cells were monitored for lymphoma development and systemic signs of illness, including apathy, breathing problems, precipitous weight loss, and limited ability to reach food or water. Animals were euthanized as soon as they exhibited any signs of illness. After subcutaneous B16 and CT26 tumor cell injection syngeneic C57BL/6 and BALB/c mice were inspected daily for tumor development. Increase in tumor size was measured with a caliper. Tumor volume was calculated as follows: (Length x width to the power of 2)/2, where L is the longer of the 2 measurements.

For antibody-mediated depletion experiments *in vivo*, mice were intraperitoneally injected with 100 μg of an anti-CD8-depleting antibody (Bioxcell, clone53-6.7, #BE0004-1) or vehicle (PBS) every second day for seven doses during 2 weeks after tumor cell injection. For antibody-mediated blockade experiments *in vivo*, mice were intraperitoneally injected with 100 μg of anti-CD86-blocking antibody (Bioxcell, clone GL-1, #BE0025) or vehicle (PBS) every second day for seven doses during 2 weeks after tumor cell injection. For dendritic cell and macrophage depletion *in vivo,* mice were injected intraperitoneally with 200 μL of a 5mg/mL clodronate-loaded liposome suspension (Liposoma B.V., #PBS-02) every second day for seven doses during 2 weeks after tumor cell injection. Control mice were injected with 200 μL PBS-loaded liposomes using the same schedule.

TUDCA (Sigma-Aldrich, #T0266) was intraperitoneally administered in PBS (250 μg/gram of mouse body weight). MKC4485 was administered by oral gavage at a dose of 10 mL/kg from a 30 mg/mL suspension in 1% microcrystalline cellulose in a simple sugar at 300 mg/kg daily (Provided by John B. Patterson). Both inhibitors were administered from day 11 until day 16 after subcutaneous tumor cell injection. Then, the mice were euthanized for further analysis. When described glycemia was measured after a few hours of fasting by using a freestyle Optium blood glucose monitoring device.

Cell Lines and Cell Culture Conditions

Lymphoma-bearing C57BL/6 Eμ-Myc mice were killed by cervical dislocation as soon as they presented signs of illness. A single-cell suspension was prepared from lymph nodes by teasing them on a 70-μm nylon filter. Cells were either resuspended in DMEM (GIBCO) supplemented with 10% fetal bovine serum (FBS), 10 mM Hepes, 0.1 mM L-asparagine, and 50 μM β-mercaptoethanol for further ex vivo analysis or reimplantation in wild-type mice. B16 cells were obtained from the ATCC (#CRL-6323) and cultured in DMEM (GIBCO) supplemented with 10% fetal bovine serum (FBS). CT26 cells were obtained from the ATCC (#CRL-2638) and cultured in RPMI-1640 medium (GIBCO) supplemented with 10% FBS and 1% sodium pyruvate. When indicated CT26 cells were cultured for 24 hr in amino acid (AA)-deprived medium (USBiological, #D9800-13). All cell lines were incubated at 37°C in a 5% CO^2 atmosphere.

METHOD DETAILS

Cytotoxicity Assay

$CD3^+$ cells were negatively sorted from mice spleens using autoMACS (Miltenyi Biotec) with FITC antibodies against CD19 (Miltenyi Biotec, #130-102-494), CD45R (Miltenyi Biotec, #130-110-845), CD49b (BD Biosciences, #553857), CD11b (BD Biosciences, #553310) and Ter-119 (BD Biosciences, #557915). The resulting purified cells were co-incubated with CT26 or B16 cells at a ratio 1:5 in the presence of IL-2 (0.1 ng/mL, AbD Serotech, #PMP38) for 4 hr or 48 hr at 37 °C. Flow cytometry (MACS-Quant Analyzer, Miltenyi Biotec) was used to analyze the cell viability of CT26 and B16 cells. CD3-negative population and back gating was used to confirm the difference in forward scatter and side scatter parameters between cells. Cell death was evaluated by looking at plasma membrane permeabilization of CT26 and B16 cells using 4',6-diamidino-2-phenylindole staining (DAPI, Sigma-Aldrich #D9542).

Flow Cytometry Analysis

To obtain a single-cell suspension from tumors, lymph nodes, and spleens were filtered through a 70-μm strainer, stained, and analyzed on MACS-Quant Analyzer (Miltenyi Biotec). The following fluorochrome-conjugated anti-mouse antibodies were used: CD4 (VioBlue, #558107), CD8 (PE, #553032), NK1.1 (PE, #557391), CD11c (PE, #557401), CD86 (PECy7, #560582), CD127 (APC, #564175), CD25 (APCCy7, #557658) (BD Biosciences). CD3 (FITC, #11-0031-85), F4/80 (VioBlue, #48-4801-82) (eBioscience).

Percentage of $CD4^+$ and $CD8^+$ cells are calculated within $CD3^+$ cells. T regs were defined as the $CD3^+CD4^+CD25^+CD127^-$ T-cell population. NK cells were defined as $CD3^-NK1.1^+$ cells. DCs were defined as $F4/80^-CD11c^+$ cells and macrophages were defined as $F4/80^+$ cells. Percentage of $CD86^+$ cells was calculated within $CD11c^+$ cells.

Confocal Laser Scanning Microscopy

Tumoral tissue was snap-frozen in O.C.T. compound (Tissue-Tek, #4583). Then, 5-μm cryosections were prepared and fixed in acetone. Purified anti-mouse CD8a (Biolegend, clone 53-6.7, #100701) was used for CD8 staining and was visualized using Alexa Fluor 594 anti-rat secondary antibody (Molecular Probes, #A11007). All sections were stained with DAPI. For each condition at least three measurements were performed. The number of CD8-positive cells was determined in optical fields of 40× on individual sections. Samples were imaged using a Nikon A1R confocal microscope and processed with ImageJ software analysis.

Western Blot Analysis

Tissue samples were collected and lysed using a Precellys 24 (Bertin Instruments) homogenizer (3 × 30 s, 6500 × *g*) in Laemmli buffer. Proteins were immunoblotted with the indicated antibodies. Immunoblots were visualized (FUJIFILM LAS4000) using the ECL Western Blotting Detection Reagents (GE Healthcare, #RPN2106), and quantification was performed using ImageJ software. anti-XBP1 (#sc-8015), and anti-ERK2 (#sc-1647) antibodies were purchased from Santa Cruz Biotechnology. Anti-IRE1α (#3294), Anti-pS6K (#9234), anti-S6K (#9202), anti-pAKT (#9271), anti-AKT (#2967), anti-LC3B (#3868), anti-eIF2α (#9722), anti-peIF2α (#9721), anti-RIG1 (#3743), anti-ATF-4 (#11815), anti-CHOP (#2895) and GCN2 (#3302) antibodies were purchased from Cell Signaling. Anti-ATF6 (#NBP1-40256) and anti-pIRE1α (#NB100-2323) were purchased from Novus Biologicals. Anti-GRP78 (#ab21685) and anti-pGCN2 (#ab75836) was purchased from Abcam.

Reverse Transcriptase Quantitative-PCR (RT-qPCR) Analysis

CT26 tumors were dissociated with the mouse Tumor Dissociation Kit (Miltenyi Biotec, #130-096-730) and $CD4^+$/$CD8^+$ TILs were sorted from the obtained single cell suspension using mouse CD4/CD8 (TIL) MicroBeads (Miltenyi Biotec, #130-116-480) in an autoMACS (Miltenyi Biotec). $CD11c^+$ DC were sorted from spleens of tumor-bearing BALB/c mice using mouse CD11c Microbeads ultrapure in an autoMACS (Miltenyi Biotec, #130-108-338). For total tumor tissue samples were collected and lysed using a Precellys 24 (Bertin Instruments) homogenizer (3 × 30 s, 6500 × *g*) and total RNA was isolated from cells and tissue using the RNAeasy minikit (Qiagen, # 74104) according to the manufacturer's protocol. Reverse transcription was performed using the Omniscript RT Kit (Qiagen, #205113). Quantitative-PCR was performed with Fast SYBR Green (Applied Biosystems, Life Technologies, # 4385616) or TaqMan Fast Universal PCR Master Mix (Applied Biosystems, #4352042) using the 7500 Fast and the Step One real-time PCR systems (Applied Biosystems) following the manufacturers' instructions.

The following primers were used for SYBR Green qPCR: *Scara3* Forward 5′-TGCATGGATACTGACCCTGA-3′ and Reverse 5′-GCCGTGTTACCAGCTTCTTC-3′; *Blos1* Forward 5′-CAAGGAGCTGCAGGAGAAGA-3′ and Reverse 5′-GCCTGGTTGAA GTTCTCCAC-3′ *Col6* Forward 5′-TGCTCAACATGAAGCAGACC-3′ and Reverse 5′-TTGAGGGAGAAAGCTCTGGA-3′ *IFNγ* Forward; 5′-TCAAGTGGCATAGATGTGGAAGAA-3′ and Reverse 5′-TGGCTCTGCAGGATTTTCATG-3′; *CXCL10* Forward 5′-GCTGATGCAGGTACAGCGT-3′ and Reverse 5′-CACCATGAATCAAACTGCGA-3′; *βactin* Forward 5′-TGGAATCCTGTGGCATC CATGAAA-3′ and Reverse 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′. *sXBP1* Forward 5′-GCTGAGTCCGCAGCAGGTG-3′; *uXBP1* Forward 5′-GAGTCCGCAGCACTCAGACT-3′ and *XBP1* Reverse 5′-GTGTCAGAGTCCATGGGAAGA-3′ (Villeneuve et al., 2010) . The housekeeping gene *β-actin* was used as a control for RNA quality and for normalization.

The following Taqman assay primer sets from Applied Biosystems were used: *Atf3* Mm00476033_m1; *Ero1lb* Mm00470754_m1; *Sars* Mm00803379_m1; *Trib3* Mm00454879_m1; *Hsp90b1* Mm00441926; *Hyou1* Mm00491279_m1. The housekeeping gene Rn18s was used as a control for RNA quality, and used for normalization: Mm03928990_g1.

All analyses were performed in triplicate, and melting curve analysis was performed for SYBR Green to control product quality and specificity.

Generation of shIFNγ- and shIRE1α-Transduced Cells

Self-inactivating viruses were generated by transient transfection of 293T cells (ATCC, #CRL-1573) and tittered as described previously (Frecha et al., 2011). Briefly For VSV-G preparation, 3 microgram of envelope plasmid was co-transfected using the classical calcium phosphate method with a 8,6 microgram Gag-Pol packaging plasmid (psPAX2, Adgene, #12260) and 8,6 microgram of a plasmid encoding a control shRNA plasmid (Santa Cruz, #sc-108060) and a self-inactivating mouse lentiviral shIFNγ plasmid (Santa Cruz, #sc-39607-SH). Eighteen hours after transfection, the medium was replaced by Opti-MEM supplemented with HEPES (Invitrogen). Viral supernatants were harvested 48 hr after transfection and filtered. The vectors were concentrated at low speed by overnight centrifugation of the viral supernatants at 3000g at 4°C.

For the generation of stable CT26 with silenced IRE1α, we used the pSUPER retroviral vector with neo+GFP (Oligoengine, #VEC-PRT-0005/0006). The target sequences were as follows: sh#1= 5′-CCAAGATGCTGGAGAGATT-3′and sh#2= 5′-GCTCGTGAATT GATAGAGA-3′. Oligonucleotides were cloned into the pSUPER vector following the manufacturer's protocol. Double-stranded DNA templates encoding siRNA oligonucleotides for IRE1α were synthesized. The specific oligonucleotide sequence contained a sense strand of 19 nucleotides followed by a short spacer (TTCAAGAGA) and the reverse complement of the sense strand. Five thymidines were added at the end of the synthesized oligonucleotide as an RNA polymerase III transcriptional stop signal. Oligonucleotides were annealed and ligated into the pSUPER vector digested by BglII and HindIII, and insertion was confirmed by EcoRI–HindIII digestion via migration in an agarose gel. For VSV-G preparation, 3 microgram of envelope plasmid was co-transfected using the classical calcium phosphate method with a 8,6 microgram MLV-Gag-Pol packaging plasmid and 8,6 microgram of

the empty pSUPER plasmid or the shIRE1α containing pSUPER plasmids. CT26 cells were transduced and sorted using a SONY sorter SH800 based on GFP expression, resulting in >95% purity.

Generation of CRISPR/Cas9 Cells

For the generation of stable CT26 with invalidated IRE1α or RIG1 cells were transfected with 3 μg of CRISPR–Cas9-expressing knockout plasmids (control, sc-418922; IRE1α, sc-429758; RIG1, sc-432915; all from Santa Cruz) using the jetPEI DNA transfection Reagent (PolyPlus Transfection, #POL101-10N) as described by the manufacturer. The knockout plasmids are a mixture of three plasmids, each carrying a different guide RNA specific for the target gene, as well as the Cas- and GFP-coding regions. GFP+ cells were selected by sorting on a SH800S Cell Sorter (Sony Biotechnology) 24 hr after transfection, and depletion of target proteins was verified by immunoblotting.

Sampling of Intracellular Metabolites

Tumor samples were resuspended in 170 μL of ultrapure water, manually crushed with a micro potter, vortexed, and then sonicated 5 times for 10 s using a sonication probe (vibra cell, Bioblock Scientific). At this step, 20μL of each sample were withdrawn for further determining the total protein concentration (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific, #23225). Then, we added 350 μL of methanol to the remaining 150 μL of lysate and we sonicated again twice for 10 s each using a sonication probe. Cell debris were then removed by centrifugation for 30 min at 4°C and 20,000g. Supernatant was recovered and incubated 1h30 on ice before a second centrifugation step for 15 min at 4°C and 20,000g. The resulting metabolic extracts were dried under a stream of nitrogen using a TurboVap instrument (Thermo Fisher Scientific) and stored at −80°C until analysis.

Dried extracts were dissolved using a given volume of 95 % mobile phase A / 5% mobile phase B to give in a 1000 ng/mL total protein concentration for alanine, arginine, proline methionine, tyrosine, isoleucine, leucine, phenylalanine, and tryptophan quantification. The extract was then diluted 2-fold for aspartic acid, glutamine, glycine, and valine quantification, while another 4-fold dilution was performed for asparagine, serine, threonine, glutamic acid, lysine, and histidine quantification. A defined concentration* of a labeled amino acids mixture 98 atom % 13C, 98 atom % 15N (Sigma-Aldrich, #608254) was added to each sample in order to normalize the signals and estimate endogenous amino acid concentrations. *13C 415N -Asn , 0.21 μg/mL; *13C3, 15N-Ser, 0.27 μg/mL; *13C4,15N-Asp, 0.64 μg/mL; *13C5,15N2-Gln, 0.24 μg/mL; *13C2,15N-Gly, 0.34 μg/mL; *13C4,15N-Thr, 0.32 μg/mL; *13C5,15N-Glu, 0.58 μg/mL; *13C3,15N-Ala, 0.54 μg/mL; *13C6,15N2-Lys, 0.26 μg/mL; *13C6,15N3-His, 0.06 μg/mL; *13C6,15N4-Arg, 0.35 μg/mL; *13C5,15N-Pro, 0.2 μg/mL; *13C5,15N-Val, 0.31 μg/mL; *13C5,15N-Met, 0.12 μg/mL; *13C9,15N-Tyr, 0.23 μg/mL; *13C6,15N-Ile, 0.26 μg/mL; *13C6,15N-Leu, 0.56 μg/mL; *13C9,15N-Phe, 0.26 μg/mL; *13C11,15N2-Trp, 0.34 μg/mL.

Analysis of Amino Acid Residues by Liquid Chromatography Coupled to High Resolution Mass Spectrometry (LC-MS)

LC-MS experiments were performed using a Dionex Ultimate chromatographic system (Thermo Fisher Scientific) coupled to an Exactive (Orbitrap) mass spectrometer (Thermo Fisher Scientific) fitted with an electrospray ion source. The mass spectrometer was externally calibrated before each analysis using the manufacturer's predefined methods and provided recommended calibration mixture. Chromatographic separation was performed on a Discovery HS F5 PFPP 5 μm, 2.1 × 250 mm column (Sigma-Aldrich) at 30°C. The chromatographic system was equipped with an on-line prefilter (Thermo Fisher Scientifics). Mobile phases were 100% water (A) and 100% aceonitrile (B), both of which containing 0.1% formic acid. Chromatographic elution was achieved with a flow rate of 250 μL/min. After sample injection (20 μL), elution started with an isocratic step of 2 min at 5% phase B, followed by a linear gradient from 5 to 100% of phase B in 18 min. These proportions were kept constant for 4 min before returning to 5% of phase B and letting the system equilibrate for 6 min. The column effluent was directly introduced into the electrospray source of the mass spectrometer, and analyses were performed in the positive ion mode. Source parameters were as follows: capillary voltage set at 5 kV, capillary temperature at 300°C; sheath and auxiliary gas (nitrogen) flow rates at 50 and 25 arbitrary units, respectively; mass resolution power of the analyzer set at 50,000 at m/z 200 (full width at half maximum, FWHM) for singly charged ions. The acquisition was achieved from m/z 50 to 250 in the positive ionization mode during the first 12 min of the run. Under these conditions, we achieved a good chromatographic separation and detection (with an average mass accuracy better than 3ppm) of the 19 targeted amino acids (under their [M+H]+ form). These species were readily identified and quantified by the isotope dilution method using 13C, 15N-labeled homologues (see above). Corresponding extracted ion chromatograms were generated and resulting peaks integrated using the Xcalibur software (version 2.1, Thermo Fisher Scientific) for alanine ([M+H]+ at theoretical m/z 90.05496, retention time 2.98 min), arginine (m/z 175.11895, 3.19 min), asparagine (m/z 133.06077, 2.81 min), aspartate (m/z 134.04478, 2.84 min), glutamate (m/z 148.06043, 2.95 min), glutamine (m/z 147.07642, 2.84 min), glycine (m/z 76.03931, 2.84 min), histidine (m/z 156.07675, 3.03 min), isoleucine (m/z 132.10191, 5.73 min), leucine (m/z 132.10191, 6.37 min), lysine (m/z 147.11280, 3.00 min), methionine (m/z 150.05833, 4.17 min), proline (m/z 116.07061, 3.22 min), phenylalanine (m/z 166.08626, 8.57 min), serine (m/z 106.04987, 2.81 min), threonine (m/z 120.06552, 2.88 min), tryptophan (m/z 205.09715, 10.58 min), tyrosine (m/z 182.08117, 5.51 min), and valine (m/z 118.08626, 3.79 min). P-values were calculated by applying a Mann Whitney test using the GraphPad Prism Software.

Bioinformatic Analysis

Patients were clustered according to IRE1α activity based on the normalized z-score of gene expression for the *BioInfoMiner* signature of 38 genes (Lhomond et al., 2018). The z-score was calculated by the equation (X - m)/s, X stands for normalized log2

expression data of each gene in each sample; m stands for mean of expression of each gene among all samples; and s stands for standard deviation. Raw data (*.CEL files) of the GSE27306 dataset (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE27306) from (Pluquet et al., 2013) were processed into R/Bioconductor by using the RMA normalization and Limma package (Ritchie et al., 2015). The deferentially expressed genes (DEGs) between DN and WT U87 cells, were selected by using a corrected p value threshold of 0.05 and fold change threshold of $|\log2(fc)| \geq 1.5$. 1051 deferentially expressed (D.E.) genes were then introduced into the *BioInfoMiner* tool and gene prioritization was executed based on the biomedical ontologies of the four-different functional and phenotype databases (GO), Reactome, MGI and HPO, separately. For the annotation process was used the ''complete'' version (this version amplifies the annotation of each gene with the ancestors of every direct correlated ontological term, exploiting the structure of ontological tree) and the hypergeometric pvalue threshold was set to 0.05. 227 highly prioritized genes including their proximal interactors was the union of the BioInfoMiner output from the four databases and 38 hub-genes were highlighted as the intersection with the IRE1α signature of 97 genes of (Pluquet et al., 2013). *The BioInfoMiner* signature was composed of 19 genes; highly up-regulated in WT versus DN U87 cells (*ASS1, C3, CCL20, COL4A6, CXCL2, CXCL5, CXCL8, IFI44L, IL1B, IL6, KCNN2, MMP1, MMP12, MMP3, PLA2G4A, PPP4R4, SERPINB2, TFPI2, ZNF804A*), and 19 genes; highly down-regulated in WT versus DN U87 cells (*ANGPT1, CFH, CFI, CLEC3B, COL3A1, COL8A1, DACH1, DCN, FHL1, GAS1, LUM, OXTR, PLAC8, RGS4, TAGLN, TGFB2, THBS1, TIMP3, TMEM255A*).

This 38-genes signature was used to stratify 3 different types of tumors including glioblastoma multiform (GBM; TCGA and GBMmark, Lhomond et al., 2018), melanoma (TCGA) and colorectal cancer (TCGA) into IRE1α high and IRE1α low activity tumors. Then based on these 2 tumor groups, the expression of the following T-cell markers was evaluated in the two groups using the transcriptome data: *IFNG, IL12, TBX21, IRF1, STAT1, GZMA, GZMB, GZMH, PRF1, GNLY, NKG7, CXCL9, CXCL10, CCL5, CX3CL1, CXCR3, CCL2, CCL4, CXCL11, MADCAM1, ICAM1, VCAM1, CD3D, CD8A, GBP1,* and all the available *HLAs*.

Statistics

Statistical analysis was conducted using GraphPad Prism 7 software. Differences in calculated means between groups were assessed by two-sided Student's t tests. For experiments involving more than two groups, differences in the calculated mean values between the groups were assessed by one-way analysis of variance (ANOVA) followed by Fisher's exact test. Kaplan-Meier survival analyses were performed, and survival curves were compared using log-rank tests. A P-value less than 0.05 was considered significant. Error bars represent the means ± standard error of the mean (SEM).

Cell Metabolism, Volume *27*

Supplemental Information

Low-Protein Diet Induces IRE1α-Dependent Anticancer Immunosurveillance

Camila Rubio-Patiño, Jozef P. Bossowski, Gian Marco De Donatis, Laura Mondragón, Elodie Villa, Lazaro E. Aira, Johanna Chiche, Rana Mhaidly, Cynthia Lebeaupin, Sandrine Marchetti, Konstantinos Voutetakis, Aristotelis Chatziioannou, Florence A. Castelli, Patricia Lamourette, Emeline Chu-Van, François Fenaille, Tony Avril, Thierry Passeron, John B. Patterson, Els Verhoeyen, Béatrice Bailly-Maitre, Eric Chevet, and Jean-Ehrland Ricci

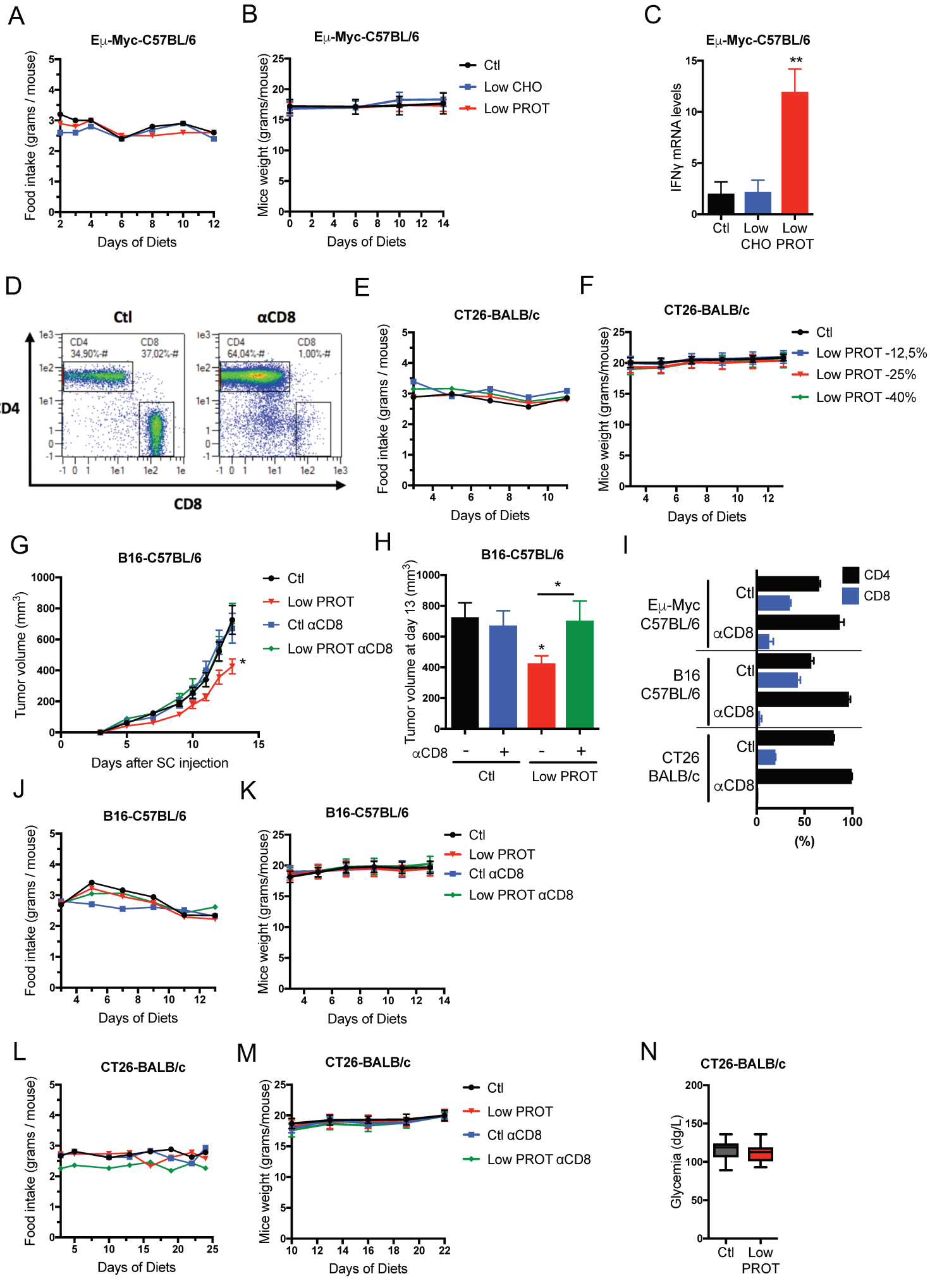


Figure S1. Related to Figure 1

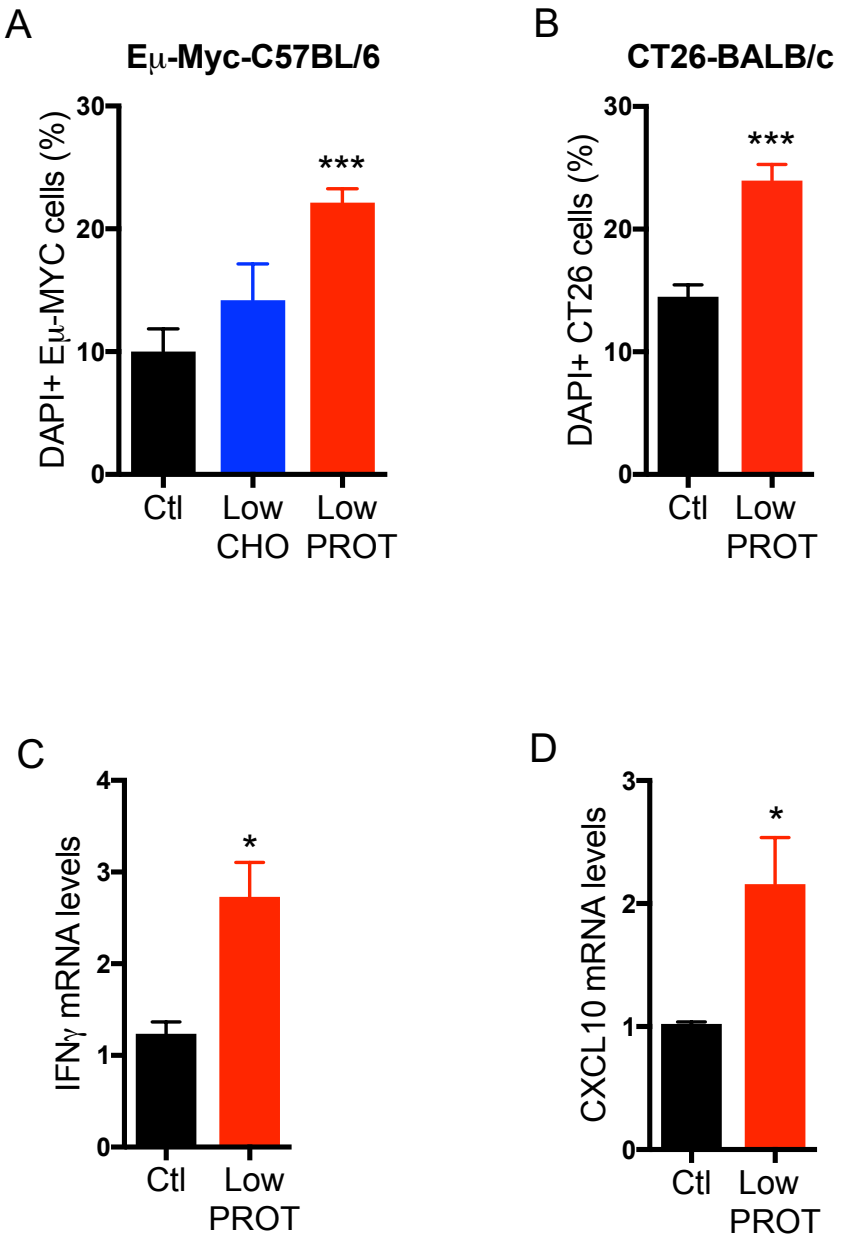


Figure S2. Related to Figure 2

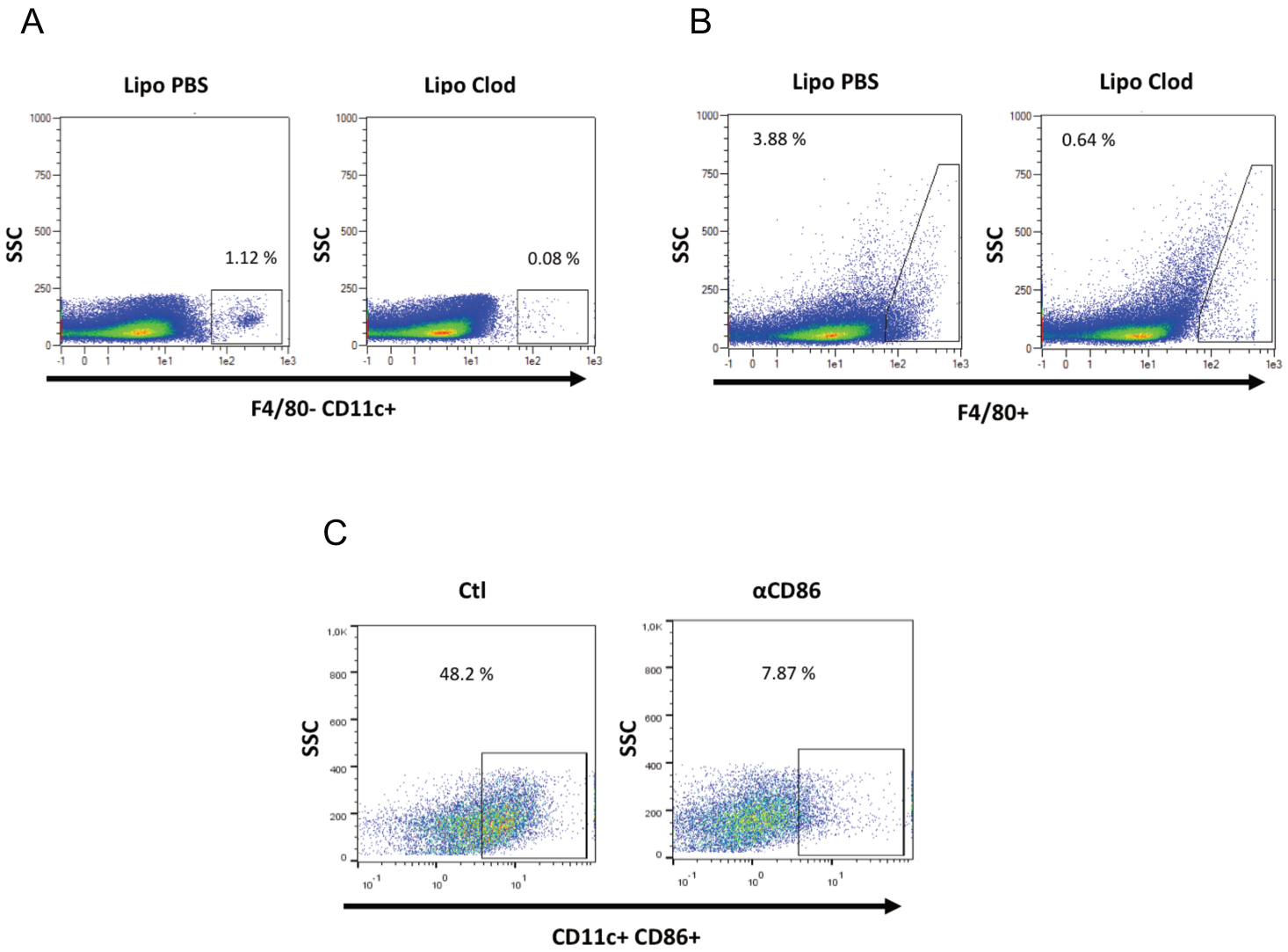


Figure S3. Related to Figure 3

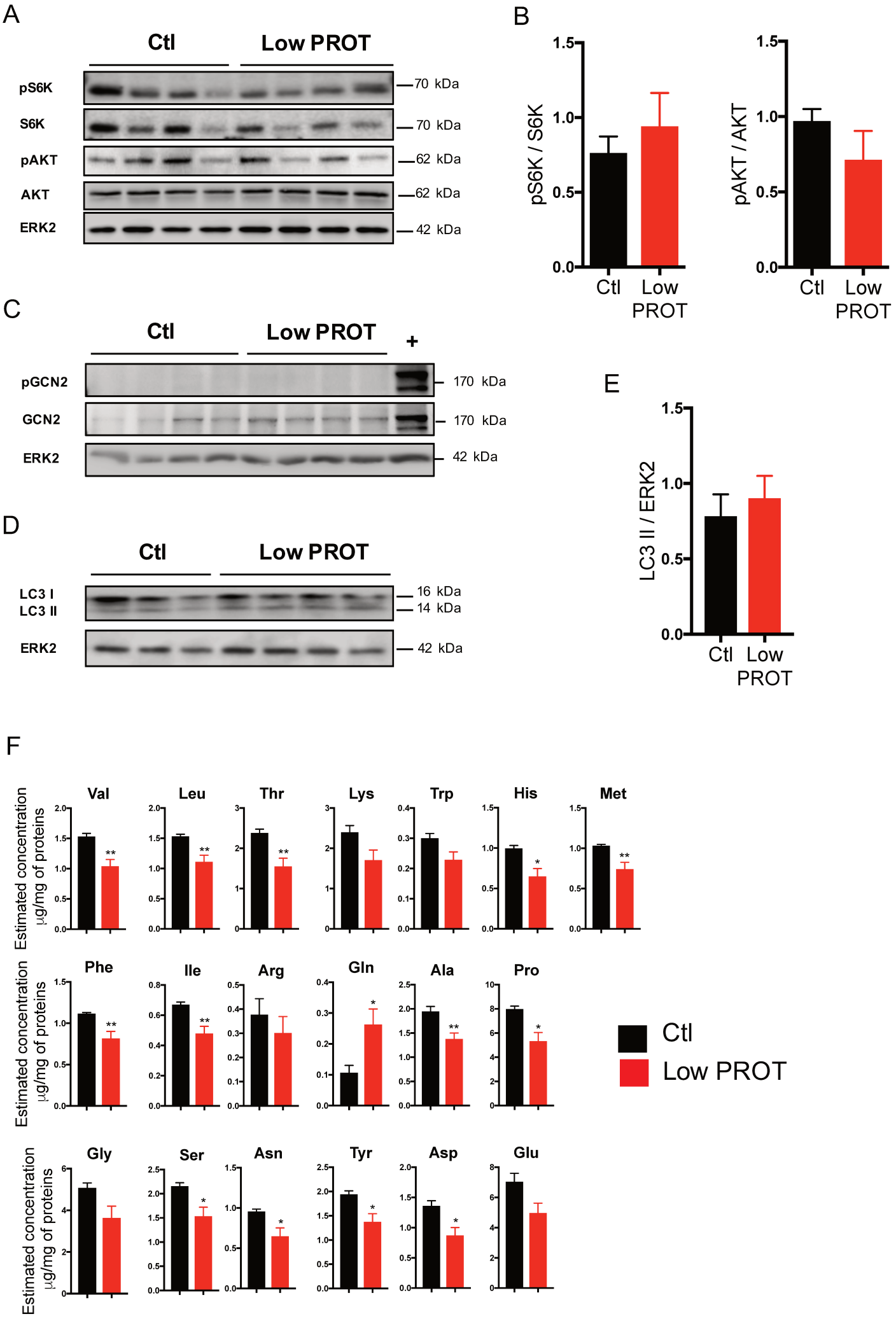


Figure S4. Related to Figure 4

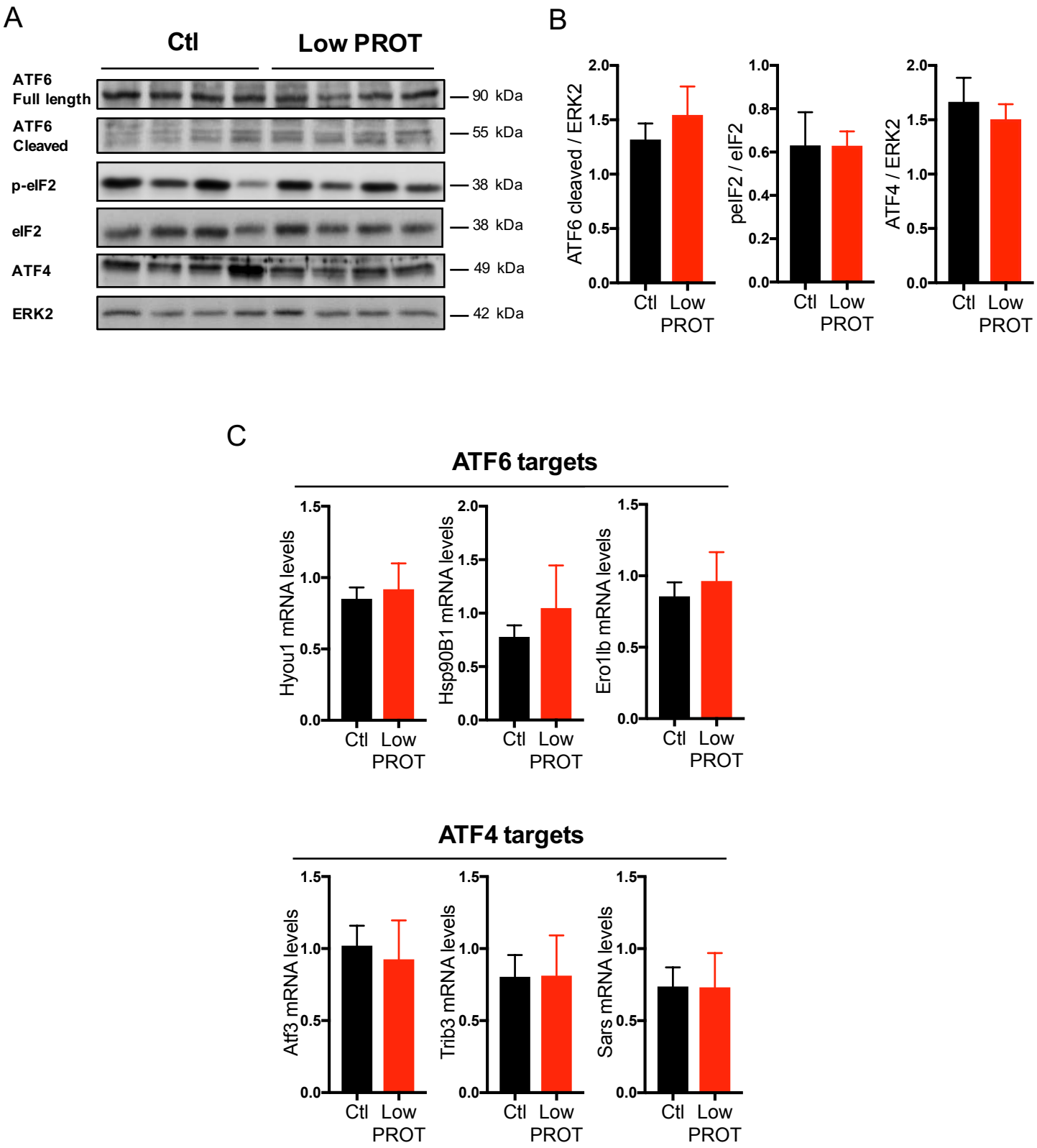


Figure S5. Related to Figure 4

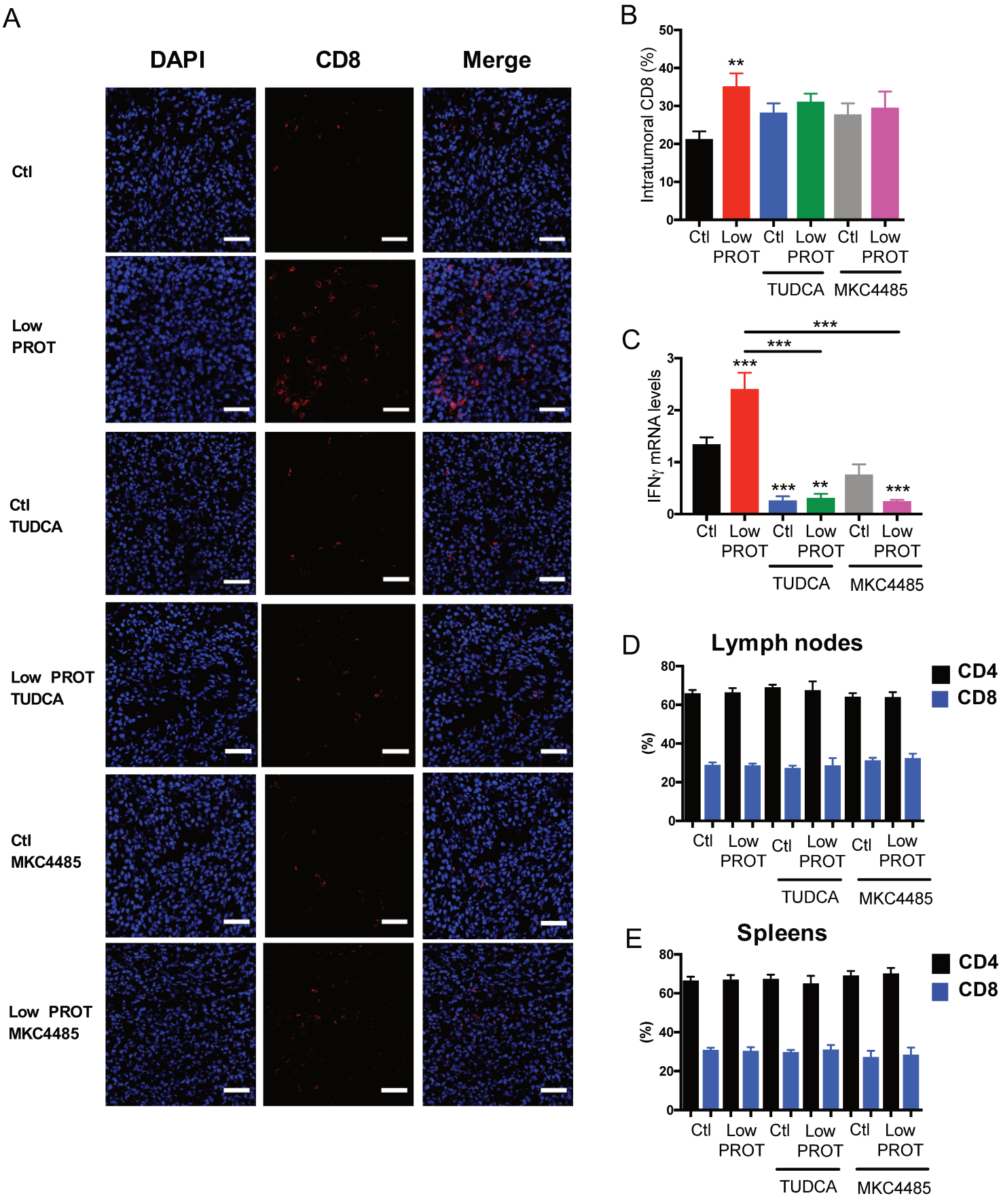


Figure S6. Related to Figure 5

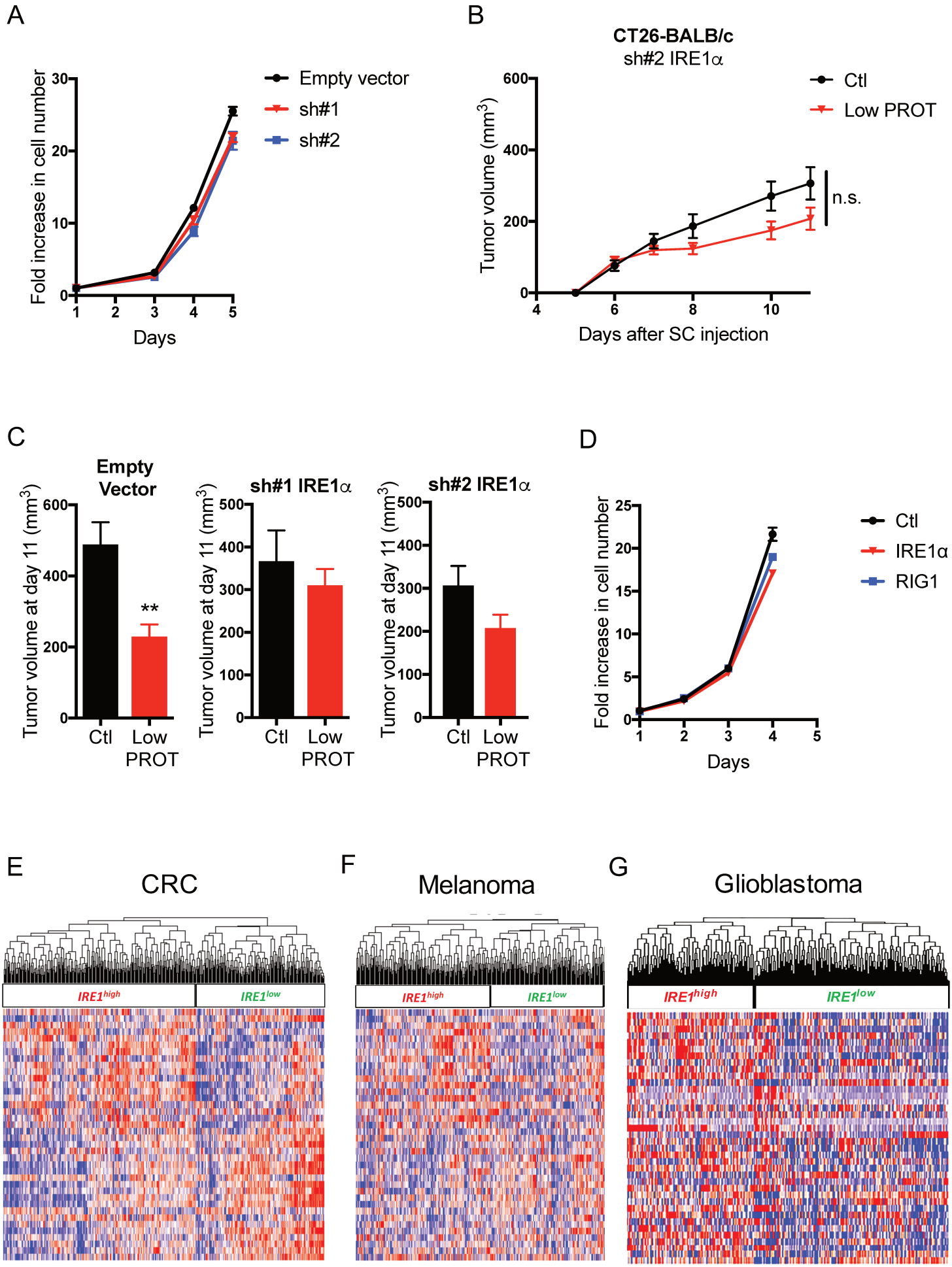


Figure S7. Related to Figure 6 & 7

Supplemental Figure legends

Figure S1. Related to Figure 1. Effect of diets on C57BL/6 and BALB/c mice injected with tumor cells. (A) Food intake and **(B)** mice weight of C57BL/6 mice injected with Eμ-Myc lymphoma cells and fed *ad libitum* with Ctl, Low CHO and Low PROT diets. **(C)** IFNγ mRNA levels in the lymph nodes of C57BL/6 lymphoma bearing mice **(D)** Flow cytometry profile showing the effective antibody-mediated depletion of CD8 T lymphocytes. **(E)** Food intake and **(F)** mice weight of syngeneic BALB/c mice that were injected with CT26 colorectal carcinoma cells and were fed *ad libitum* with Ctl and Low PROT (-12,5%, -25% and -40%) diets. **(G)** Tumor growth curve of syngeneic C57BL/6 mice that were subcutaneously injected with B16 melanoma cells. Mice were fed *ad libitum* with Ctl and Low PROT diets and were intraperitoneally injected with PBS or αCD8 antibody (Ctl, n=6; Low PROT, n=8; Ctl αCD8, n=6; Low PROT αCD8, n=8). Tumor volume at day 12 **(H)** of data shown in **(G)**. **(I)** Confirmation of antibody-mediated depletion of CD8 T lymphocytes in mice. Flow cytometry analysis of the proportion of CD4 and CD8 T cells in spleens of C57BL/6 mice injected with Eμ-Myc or B16 cells and BALB/c mice injected with CT26 cells. **(J)** Food intake and **(K)** mice weight of syngeneic C57BL/6 mice that were injected with B16 colorectal carcinoma cells. **(L)** Food intake and **(M)** mice weight of syngeneic BALB/c mice that were injected with CT26 colorectal carcinoma cells. Mice were fed ad libitum with Ctl and Low PROT diets. **(N)** Glycemia was measured in BALB/c mice that were injected with CT26 colorectal carcinoma cells after 21 days of Ctl and Low PROT diets. *P < 0.05, **P < 0.01. Error bars represent SEM. When not mentioned, differences are not significant. Comparisons of every group vs. Ctl group, except where specified otherwise. When not mentioned, differences are not significant.

Figure S2. Related to Figure 2. A Low PROT diet induces IFNγ and CXCL10 mRNA levels in CT26 tumors. (A-B) C57BL/6 mice were intravenously injected with Eμ-Myc cells and BALB/c mice were subcutaneously injected with CT26 colorectal carcinoma cells. C57BL/6 mice were fed *ad libitum* with Ctl, Low CHO and Low PROT diets. BALB/c mice were fed with Ctl and Low PROT diets. Upon sacrifice, $CD3^+$ cells were isolated from spleens and incubated

with live Eμ-Myc cells or CT26 cells for 48 hours. The ability of T cells to kill tumor cells was determined by flow cytometry. Cell death of Eμ-MYC **(A)** and CT26 cells **(B)** was determined by DAPI staining (3 mice per group). **(C-D)** BALB/c mice that were subcutaneously injected with CT26 colorectal carcinoma cells and were fed ad libitum with Ctl and Low PROT diets. Tumors were harvested and **(C)** IFNγ and **(D)** CXCL10 mRNA levels were measured by qPCR (at least 3 mice per group). *P < 0.05, ***P < 0.001. Error bars represent SEM. Comparisons of every group vs. Ctl group, except where specified otherwise. When not mentioned, differences are not significant.

Figure S3. Related to Figure 3. Effect of clodronate liposomes and CD86 blockade on myeloid cells. BALB/c mice were subcutaneously injected with CT26 colorectal carcinoma cells and were fed *ad libitum* with Ctl and Low PROT diets. **(A)** Mice were intraperitoneally injected with liposomal clodronate (Lipo Clod) or PBS liposomes (Lipo PBS). Flow cytometry profile confirming Lipo Clod-mediated depletion of dendritic cells ($F4/80^-$ $CD11c^+$) and macrophages ($F4/80^+$). **(B)** Flow cytometry profile showing the effective antibody-mediated blockade of CD86 on $CD11c^+$ cells.

Figure S4. Related to Figure 4. A Low PROT diet does not modulate mTOR, AKT, GCN2 or autophagy in CT26 tumors. Syngeneic BALB/c mice were subcutaneously injected with CT26 colorectal carcinoma cells and fed *ad libitum* with Ctl, and Low PROT diets. **(A)** Tumors were harvested after 25 days of diets and lysates were prepared. Expression of indicated proteins were analyzed by western blot. **(B)** Average quantification of pS6K and pAKT compared with the corresponding total protein levels. **(C)** Expression of pGCN2 and the corresponding total protein was analysed by western blot. (+) corresponds to CT26 cells cultured under amino acid deprivation conditions, used as a positive control for GCN2 activation. **(D)** Status of LC3 autophagy related conversion was analyzed by immunoblots. **(E)** Average quantification of LC3II compared with ERK2 levels (used as a loading control). **(F)** relative amount of amino acids measured in CT26 tumors isolated from Ctl or Low PROT diet fed

mice (n=8 for Ctl and n=7 for Low PROT condition). Error bars represent SEM. When not mentioned, differences are not significant.

Figure S5. Related to Figure 4. A Low PROT diet does not modulate ATF6, eIF2 or ATF4 in CT26 tumors. **(A)** Expression of ATF6, eIF2 and ATF4 proteins were analyzed by immunoblots. **(B)** Average quantification of cleaved ATF6, peIF2 and ATF4 compared with ERK2 levels (used as a loading control) or the corresponding total protein. **(C)** mRNA levels of ATF6 and ATF4 targets were measured in tumors by qPCR (at least 3 mice per group). Error bars represent SEM. When not mentioned, differences are not significant.

Figure S6. Related to Figure 5. Treatment with ER stress inhibitors affects the recruitment of CD8 Tumor Infiltrating Lymphocytes. BALB/c mice that were subcutaneously injected with CT26 cells and were fed ad libitum with Ctl and Low PROT diets. Mice were treated with TUDCA and MKC4485 from day 11 to 16 after subcutaneous injection. **(A)** Immunofluorescent staining of CD8 T cells in tumors, scale bar is equivalent to 50 µm **(B)** Flow cytometry analysis of the frequency of infiltrating CD8 T cells in CT26 tumors. **(C)** IFNγ mRNA levels in CT26 tumors of BALB/c mice. **(D)** Flow cytometry analysis of the proportion of CD4 and CD8 T cells in lymph nodes and **(E)** spleens of BALB/c mice. **P < 0.01, ***P < 0.005. Error bars represent SEM. Comparisons of every group vs. Ctl group, except where specified otherwise. When not mentioned, differences are not significant.

Figure S7. Related to Figure 6 & 7. Knockdown in CT26 cells of IRE1α using two different shRNAs. **(A)** Proliferation curves in vitro. **(B)** Tumor growth curve of BALB/c mice that were subcutaneously injected with sh#2 IRE1α CT26 cells and were fed ad libitum with Ctl and Low PROT diets. **(C)** Tumor volume at day 11 of data shown in Fig 6B-C and Sup Figure 7B. **(D)** Proliferation curves in vitro of CRISPR Ctl, CRISPR IRE1α and CRISPR RIG1 CT26 colorectal carcinoma cells. Hierarchical clustering of CRC (n=456; **E**), Melanoma (n=293; **F**) and Glioblastoma (n=523, **G**) patients (TCGA cohorts) based on high or low

IRE1α activity as assessed with the median z-score of the expression pattern of the IRE1α gene signature of 38 hub-genes (Lhomond et al. 2018). Pearson correlation was used to measure the similarity between different genes and tumor cases, as well. The expression pattern of WT vs. DN has been described in detail in (Pluquet et al., 2013). Blue: low mRNA expression levels, Red: high mRNA expression levels. Error bars represent SEM. When not mentioned, differences are not significant.

2. Preliminary result

IRE1 activation correlates with increased MHC-I expression.

We have previously showed that low PROT diet in mice induces an anti-tumor immune response dependent on specific IRE1 signaling (Rubio-Patino, Bossowski et al., 2018b). Mechanistically, low PROT diet induced immunosurveillance by activation of an IRE1/RIG1 axis within the tumoral cells, concomitant with enhanced cytokine expression and $CD8^+$ T cell-dependent anti-cancer cytotoxic response. Although increase in cytokine secretion is required for enhanced immune response, other molecular modifications that remains to be identified are required for the induction of specific anti-cancer immune response. As IRE1 signaling in tumor cells seems to be the core determinant of low PROT-induced immunosurveillance, we focused our attention on the effect that modulation of this pathway could have on the immunogenic phenotype of cancer cells. A main determinant of effective adaptive immune response and tumor immune escape mechanism is the modulation of surface expression of MHC-I molecules (Garrido, Aptsiauri et al., 2016). As MHC-I is a critical determinant of cancer cell immunogenicity, we aimed to analyze the effect of IRE1 on MHC-I surface expression. In this regard, we used CT26 cells invalidated for IRE1 by CRISPR/Cas9 technology. Surprisingly, we observed lower surface expression of MHC-I in IRE1-deficient CT26 cells as compared to control cells (Fig 1A).

We reasoned that if IRE1 deficiency leads to downregulation in MHC-I surface expression, then IRE1-specific induction could conversely lead to MHC-I increase. As there is no known specific inducer of IRE1 which is available, and the commonly used ER stress inducing agents provoke general UPR induction (not specific to IRE1), we decided to use BSA-conjugated palmitate (PA/BSA) as one of the specific IRE inducer reported so far, as some studies reported that lipid alterations affecting ER membrane lipidic composition could more directly induce IRE1 (Kitai, Ariyama et al., 2013, Lancaster, Langley et al., 2018) (see Fig. 6 for a proposed model). We chose 100 µM dose of PA/BSA, which over 24-hour treatment did not affect cell viability (Fig. 1B) and was reported to induce IRE1 activation (Kitai et al., 2013, Lancaster et al., 2018). We performed a kinetic assay of PA/BSA treatment over 24-hour period in CT26 cells, where we analyzed UPR activation and MHC-I surface expression. Tunicamycin (1 µg/mL) treatment was used as a positive control for general ER stress induction. We observed a time-dependent increase in phosphorylation

of IRE1 upon PA/BSA stimulation, starting at 1hr and lasting over 24hr-period as assessed by western blot (Fig. 1B). In contrast, other branches of UPR were not, or only mildly affected, as assessed by ATF4 expression and ATF6 cleavage (Fig. 1C). To determine the induction of IRE1 RNase activity, we measured the relative levels of spliced mRNA XBP1 both by classical PCR and by real time qPCR (Fig. 1 D-E). In both approaches, we observed strong time-dependent induction of XBP1 splicing, indicating IRE1 RNase activation. Finally, we analyzed the surface expression of MHC-I by flow cytometry considering only viable CT26 cells in the kinetic treatment with PA/BSA, where we observed a modest but consistent increase in MHC-I levels over a 24hr-period, contrary to cells treated with tunicamycin, where MHC-I levels potently declined (Fig. 1F). Given that tunicamycin also stimulated IRE1 RNase activity (Fig.1 D-E), we concluded that probably additional mechanisms induced by a more general ER stress leads to MHC-I decrease, whereas the specific IRE1 activation drives MHC-I overexpression on the cell surface. It remains to be determined if the PA/BSA treatment of IRE1 invalidated CT26 does not modulate MHC-I expression. As the effect of PA/BSA and tunicamycin on MHC-I surface expression are in strike contrast, we cannot exclude the participation of additional signaling pathways in this phenomenon, as under tunicamycin treatment we cannot distinguish between the effects of other branches of the UPR. Nevertheless, as UPR activation is usually reported as a negative regulator of MHC-I expression (Ulianich, Terrazzano et al., 2011), we consider our results linking IRE1 activity with positive regulation of MHC-I expression of high relevance.

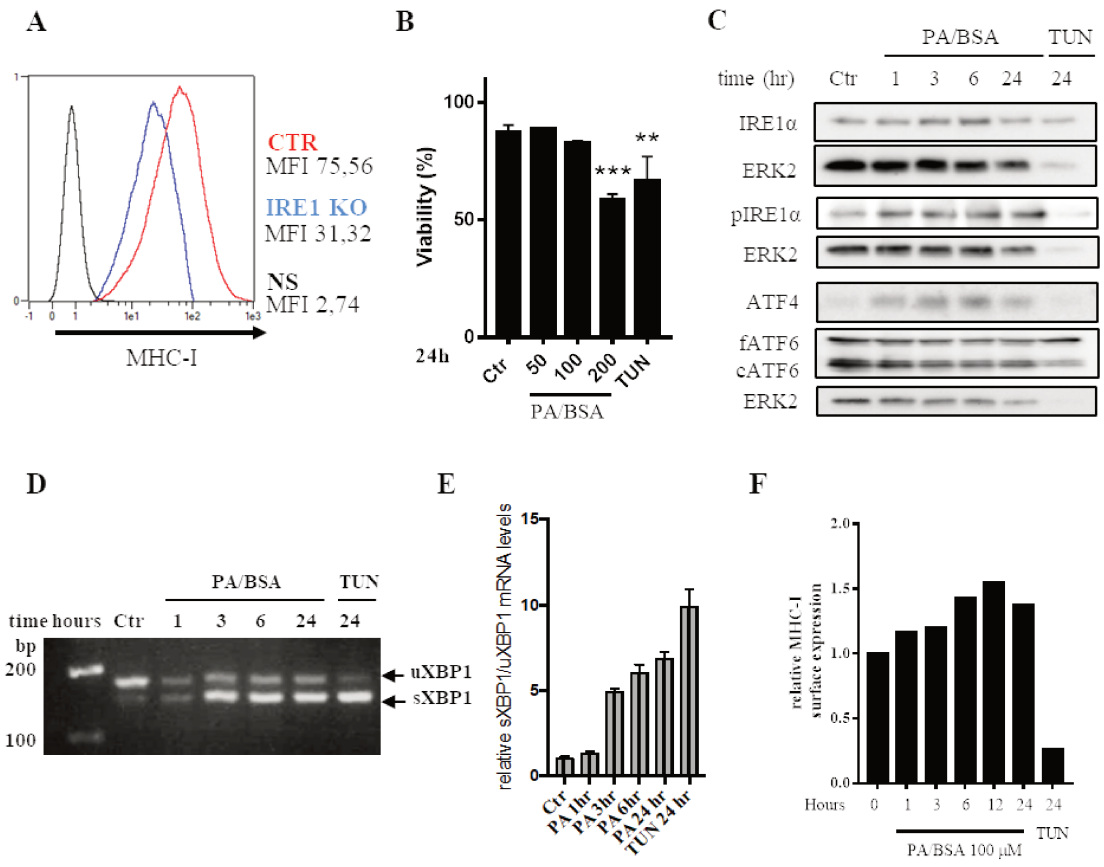


Figure 1. Palmitate induces IRE1 activation in CT26 colorectal cancer cells. **(A)** Histograms of MHC-I expression in CT26 CRISPR CTR and IRE1 KO cells as assessed by flow cytometry. **(B)** Viability of CT26 cells treated with BSA-conjugated palmitic acid (PA). **(C)** Western blot and **(D)** PCR analysis reveal activation of IRE1α upon PA stimuli over time. **(E)** Relative sXBP1 mRNA expression respect to uXBP1 in CT26 cells treated with palmitic acid as assessed by qPCR analysis. **(F)** Surface expression of MHC-I in PA/BSA-treated-CT-26 cells over time as assessed in one representational experiment by flow cytometry. PA/BSA (100μM), Palmitic Acid conjugated with BSA; TUN, (1 ug/mL) Tunicamycin.

Glutaminase inhibition induces IRE1 activation and MHC-I surface expression.

Although it is reported that lipid modulation can induce specific IRE1 membrane clustering and activation (Kitai et al., 2013), and the treatment with BSA-Palmitate *in vitro* indeed resulted in such activation in CT26 cells (Fig. 1 and Fig. 6), we have no direct evidences that lipid profile differs among tumors isolated from CTR and low PROT diet fed-mice. Instead, we observed a modulation in amino acid levels among tumors isolated from mice fed CTR and low PROT diet, with particular decrease in the glutamate/glutamine ratio (Article 1 Fig. 6). However, we do not know if this glutamine/glutamate modulation is the result of altered cellular metabolism or if it is the result of differential extracellular glutamine uptake. There are evidences that fatty acid metabolism can be affected by the disruption of glutamine/glutamate conversion, which in turn modulates lipid composition, but this has yet to be tested in our model of low PROT diet (Biancur, Paulo et al., 2017, Halama, Kulinski et al., 2018). Surprisingly, the inhibition of glutaminase has been reported to upregulate the proteins involved in lipid and fatty acid-related processes (Biancur et al., 2017). Thus, the result of glutaminase inhibition on lipid-driven IRE1 modulation is not evident, and has to be experimentally tested. For that matter, and to more directly translate the conditions of glutamine/glutamate modulation that we observed *in vivo*, we decided to investigate whether glutamine metabolism could impact IRE1 activity and in turn MHC-I expression, as we observed with PA/BSA treatment. We treated CT26 cells with a specific glutaminase 1 inhibitor, CB-839, known to block the conversion of glutamine to glutamate, the first enzymatic conversion enabling glutamine to enter cellular metabolic pathways such as the TCA (Gross, Demo et al., 2014). First, we established the treatment dose of 10 µM CB-839, as that level did not affect cell viability, but impacted in cellular stress, reflected in diminished cell proliferation (Fig. 2A). Subsequently, we treated CT26 cells with CB-839 for 24 hour and analyzed the surface expression of MHC-I. As shown in Fig. 2B, glutaminase inhibition resulted in increased MHC-I expression resembling PA/BSA effect, as contrary to tunicamycin treatment. To assess IRE1 activation, we analyzed the generation sXBP1 by qPCR (Fig. 2C) and phosphorylation of IRE1 by western blot (Fig. 2D), where in both cases we observed a potent IRE1 activation and RNAse-dependent signaling. Strikingly, CB-839 did not produced significant increase in CHOP expression, as compared to complete glutamine deprivation in the medium (Fig. 2E), indicating that IRE1 activation is more specifically induced by glutaminase inhibition than by glutamine deprivation.

This uneven induction of CHOP between CB-839 and glutamine deprivation could be a result of activation of nutrient-sensing pathways, triggered by glutamine deprivation, which might not be the case when glutamine is still present, but its metabolic conversion is blocked.

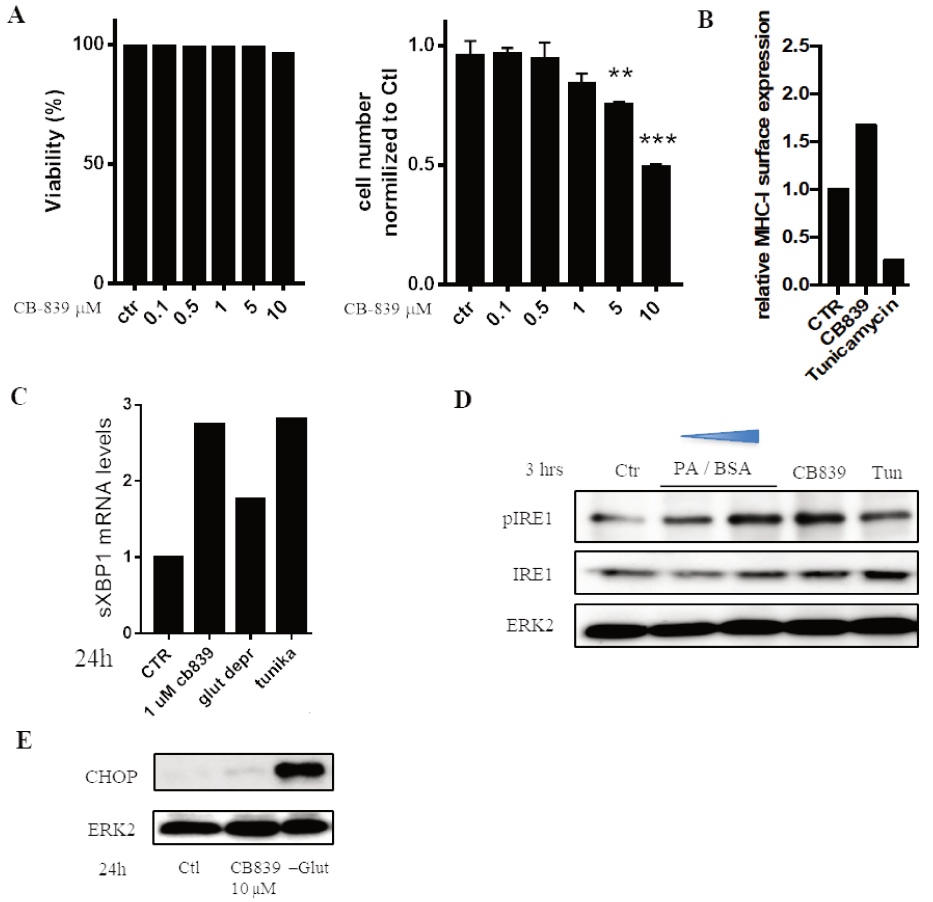


Figure 2. Glutaminase inhibition induces IRE1 activation and MHC-I surface expression in CT26 cells. **(A)** Viability and cell number of CT26 cells treated with CB-839 glutaminase inhibitor for 24h as estimated by $DAPI^-$ flow cytometry staining. **(B)** MHC-I surface expression in CT26 cells under treated with CB-839 and tunicamycin for 24h as assessed by flow cytometry **(C)** Relative sXBP mRNA expression respect to uXBP1 in CT26 under CB-839 treatment, glutamine deprivation or tunicamycin (tunika) treatment as assessed by qPCR analysis. **(D)** Western blot analysis of CT26 cells treated with BSA-Palmitate (PA/BSA), glutaminase inhibitor CB839 and tunicamycin (Tun) for 3h **(E)** Western blot analysis of CT26 cells treated with glutaminase inhibitor CB839 or glutamine deficient media (-Glut) for 24h.

Low-PROT diet reduces immune checkpoint markers and induces IFN expression.

Immune checkpoints are immunosuppressive markers hampering effective anti-cancer immune response. There is a huge interest in developing clinical treatments targeting immune checkpoints signaling to reactivate compromised immune response. For example, recent studies showed enhanced antitumor immune response with the use of the calorie restriction mimetic metformin via downregulation of PD-L1 expression in tumor cells (Cha, Yang et al., 2018). We therefore investigated expression of immune checkpoint *in vivo* upon low-PROT diet regimen. For that matter we first reproduced our findings of low PROT-driven tumor growth suppression (Fig. 3 A-B). We observed the expected increase in TILs under low-PROT regimen (Fig. 3C). Interestingly, the expression of immunoinhibitory checkpoint markers PD1 and CTLA4 were significantly decreased in $CD8^+$ T lymphocytes present in tumors of mice fed low-PROT diet, as compared to those on the control diet (Fig 3C). That decrease in immunoinhibitory signaling could contribute to enhanced anti-cancer immune response and tumor growth inhibition under low-PROT conditions. Additionally, PD-L1 decrease was specific to $CD8^+$ sub-population, as we did not observe differences in PD-L1 expression within $CD45^+$ cells, which is the pan-marker of lymphocytes. Moreover, we found increased gene expression of *IFNα* and a trend toward increased levels of *IFNβ* and *IFNγ* in whole tumor lysates. In conclusion, low-PROT diet reduced immune checkpoint surface expression on central anti-cancer cytotoxic T lymphocytes and simultaneously increased IFN type I signaling.

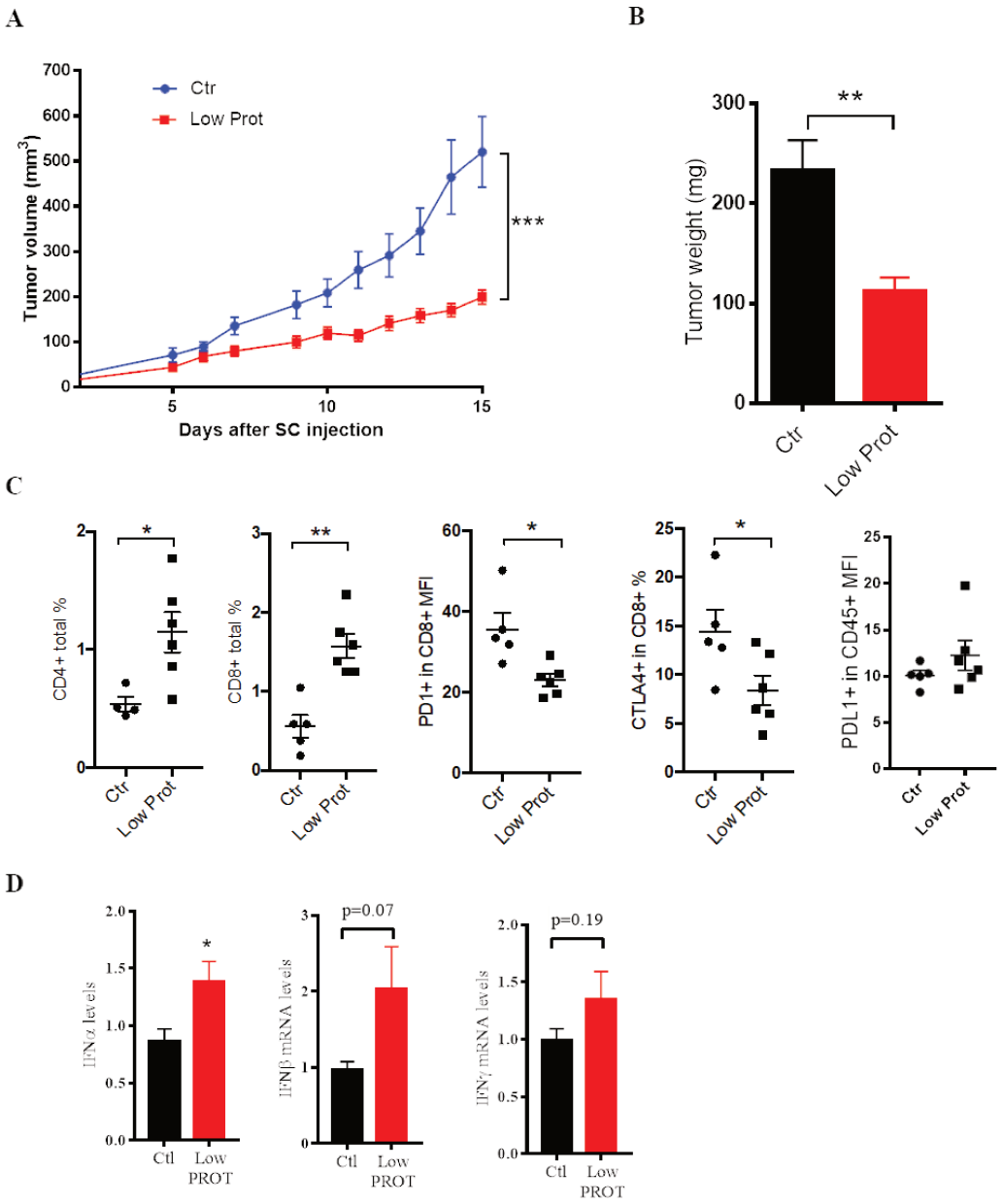


Figure 3. Low PROT diet downregulates immune checkpoints on CD8$^+$ lymphocytes in CT26/BALBc colorectal cancer mouse model. **(A)** Tumor growth and **(B)** weight at the endpoint of CT-26 tumor bearing-BALB/c mice fed CTR and Low PROT diets (Ctr: n=12 , Low PROT: n=11) **(C)** Flow cytometry analysis of immune cell populations within the tumors and surface expression of inhibitory immune markers. **(D)** Gene expression analysis of cytokines in whole tumor extracts isolated from tumors in A, at least 3 per group. *P < 0.05, **P < 0.01. Error bars represent SEM.

Low-PROT diet impacts on cancer cell immunogenic phenotype.

To directly address the phenotype of cancer cells under low PROT diet regimen, we negatively sorted the tumor cell population from CT26 tumor-bearing mice shown in Figure 3. First, we observed modulation in surface expression of cell markers regulating the immune response: NKG2D ligand H60, *don't eat me* marker CD47 and immune checkpoint marker PD-L1 as measured by flow cytometry (Fig. 4A). Importantly, tumor cells isolated from low PROT diet-fed mice exhibited markedly elevated MHC-I expression as compared to those in CTR diet, accompanied by significant increase in CD47 and PD-L1 expression. In contrast, expression of H60, a MHC-I-like glycoprotein which is a known as a NKG2D ligand, was reduced under low PROT diet, and CTLA4 expression was not modulated (Fig. 4A). These results reveal complex regulation of the surface immune marker composition on tumor cells under low PROT diet feeding as compared to CTR diet. Next, we measured the mRNA level of a panel of cytokines, chemokines and inflammatory-related genes in sorted tumor cells (Fig. 4B). The levels of the cytokines *IFNα*, *IFNβ*, *CXCL1*, *CXCL10*, *CXCL11*, *IL-15* and *GM-CSF* were increased under low PROT diet, as well as interferon-stimulated gene 15 (*ISG15*) and the chemokine *CCL2* (Fig. 4B). To further extend our observations, we measured the expression of genes implicated in MHC-I assembling machinery, to assess whether the modulation of MHC-I expression is driven at the transcriptional level (Fig. 4C). Indeed, we have found increased levels of *TAP1* gene, whose protein product is important in MHC-I assembling (Blees, Reichel et al., 2015). Additionally, we found elevated *TRIM69* mRNA, E3 ubiquitin ligase implicated in immune tumor control and apoptosis. We also investigated gene expression of known mouse NKG2D ligands, and we found that only one of them, *RAE-I* was significantly upregulated under low PROT diet (Fig. 4D). That was unexpected, as we have not found any sign of *in vivo* and *in vitro* surface expression of this marker by flow cytometry. It is possible that as soon as RAE-1 is exposed on the plasma membrane surface, the cell is instantly recognized and eliminated by surrounding immune cells. The other explanation would be that *RAE-1* is mutated and even though it is transcribed, the protein is not functional. However, this hypothesis has to be tested in future experiments.

Collectively, our data indicate that feeding tumor-bearing mice a low-PROT diet results in alteration in the cell secretome and cancer immunogenicity. Whether these two events are

reciprocal or independent has to be yet determined. Although, it is likely that modulation in cytokine secretion would eventually impact on surface marker expression, such as positive regulation of IFNγ on MHC-I expression. Furthermore, it is not clear if any of the described

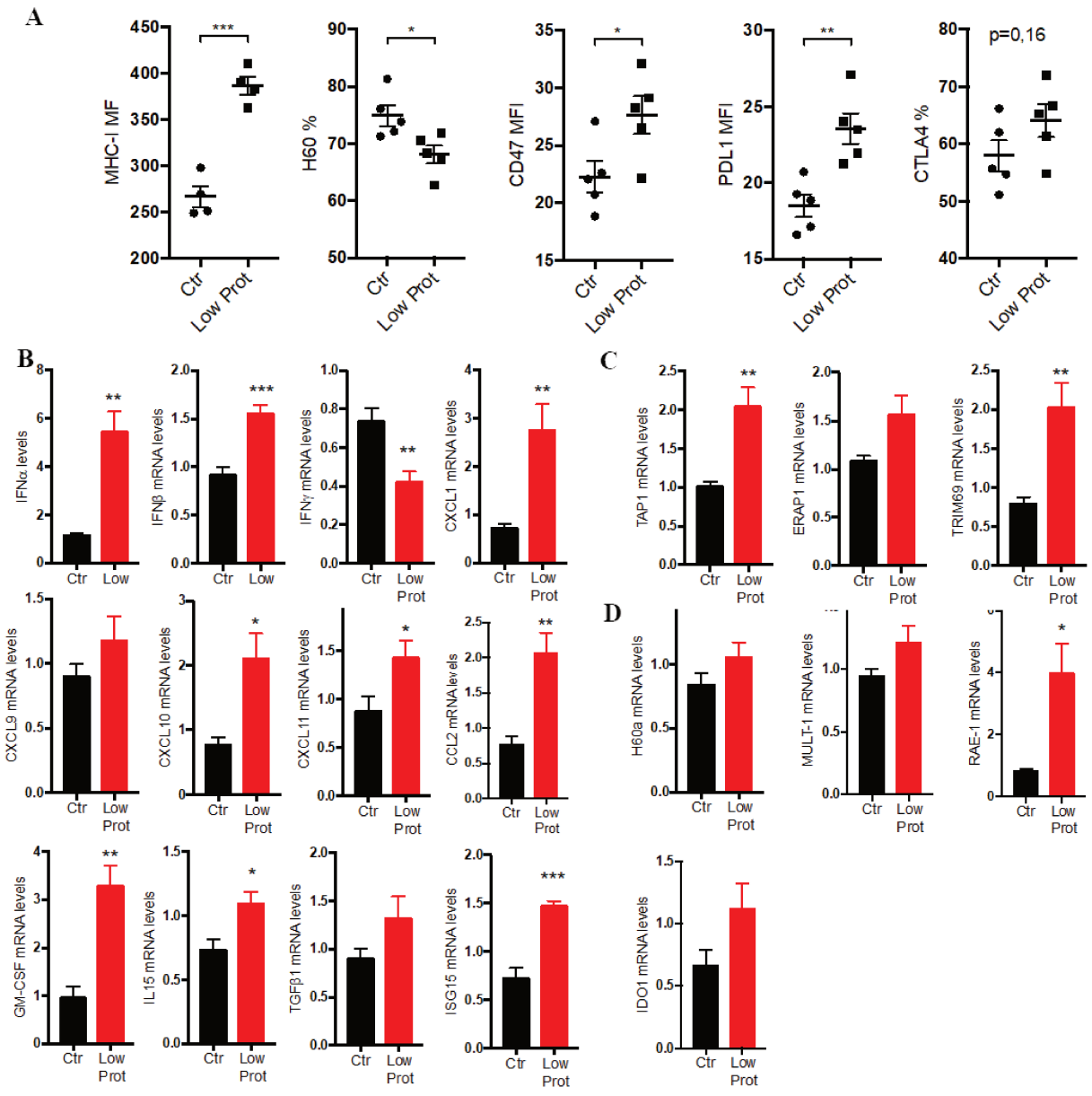


Figure 4. Flow cytometry and qPCR analysis of isolated CT26 cells from CT26 tumor-BALB/c mice under CTR and Low PROT diets. (A) Flow cytometry analysis of surface expression markers in CT26 cells **(B)** Gene expression analysis of cytokines and chemokines in sorted CT26 tumor cells. At least 3 mice per group. **(C)** Gene expression analysis of MHC-I assembling machinery. **(D)** Gene expression analysis of NK-G2D ligands and IDO1 in isolated tumor cells. *P < 0.05, **P < 0.01, ***P<0.001. Error bars represent SEM.

modulation in cytokines, chemokines or surface immune markers are dependent on the IRE1-RIG-I axis induced by low PROT diet.

Low-PROT diet promotes Retinoblastoma protein phosphorylation

As we observed some modulation in NKG2D ligands expression on cancer cell under different diet regimens, we speculated what could be the mechanism regulating such modulation. E2F family of proteins implicated in the control of cell cycle has been described as regulators of some NKG2D ligands expression (Jung, Hsiung et al., 2012). Thus, we tested the expression of E2F1 and phosphorylation levels of retinoblastoma (RB) protein in whole tumors by western blot analysis. We found no changes in E2F1 protein levels, but we observed elevated phosphorylation of RB under low PROT diet regimen (Fig. 5). RB protein can bind transcription factors such as members of the E2F family and thereby inhibit their functions. Phosphorylation of RB disrupts that this interaction and releases E2F transcription factors, hence promoting their activity. These data suggest that E2F proteins might have higher activity via lower inhibitory regulation of RB in tumors from Low PROT diet fed mice. Interestingly, this result is in opposition with what we might expect, since we observed a reduction in tumor growth under Low PROT diet, and phosphorylation of RB protein is associated by cell proliferation. However, if the immune response induced by low PROT diet is signaled via NKG2D ligands (at least partially), the observed phosphorylation of RB could play a role in mediating enhanced anticancer immune response. Future experiments will address the link between Low PROT-induced immune response and cell cycle control in tumor cells.

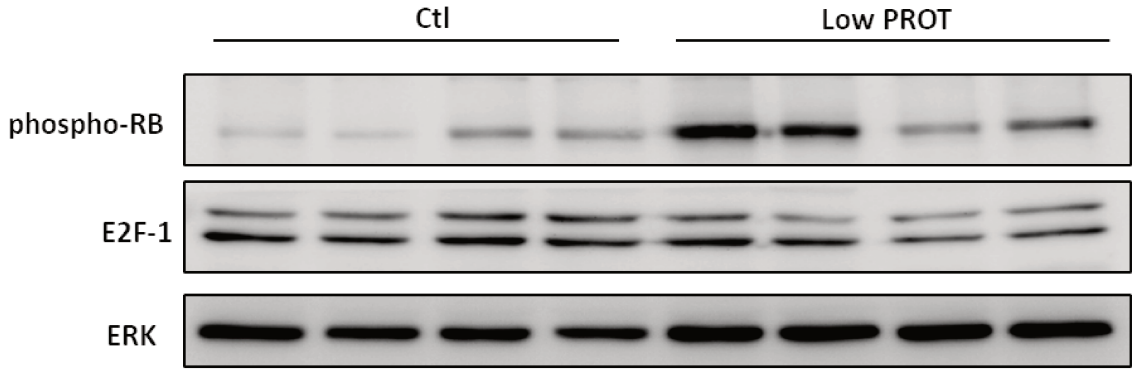


Figure 5. A Low PROT diet increase phosphorylation of Retinoblastoma (RB) protein in CT26 tumors. Syngeneic BALB/c mice were subcutaneously injected with CT26 colorectal carcinoma cells and fed *ad libitum* with CTR, and Low PROT diets. Tumors were harvested after 25 days of diets and lysates were prepared. Expression of indicated proteins were analyzed by western blot.

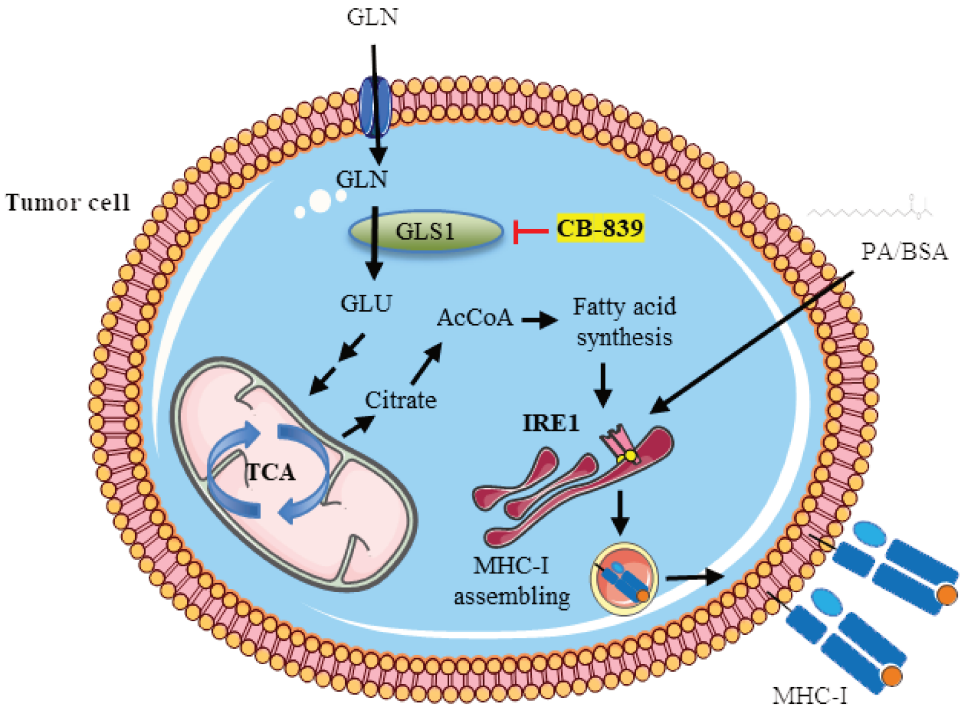


Figure 6. A recapitulative scheme of glutamine metabolism and IRE1 activation. First step of glutamine incorporation to cellular metabolism is its conversion to glutamate by GLS1. Glutamate is being subsequently processed and can enter mitochondrial to fuel TCA cycle, acting as an anaplerotic precursor. Citrate can exit TCA cycle and enhance synthesis of AcCoA, central metabolite participating in fatty acid biosynthesis. The modulation of ER lipid composition can trigger IRE1 activation, which in turn control cell secretome, including MHC-I assembling machinery and its components. Direct treatment with saturated fatty acid Palmitate (PA/BSA) can induce IRE1 activation. AcCoA, acetyl-CoA; GLN, glutamine; GLU, glutamate; GLS1, glutaminase 1; TCA, tricarboxylic acid cycle; PA/BSA, BSA-conjugated palmitate; IRE1, Inositol Requiring Enzyme 1; MHC-I, major histocompatibility complex class I.

DISCUSSION AND PERSPECTIVES

We have demonstrated that IRE1 expression in tumoral cells is critical in orchestrating an effective anti-cancer immune response induced by low-PROT diet in three independent syngeneic mouse models: Eμ-myc lymphoma, colorectal carcinoma and melanoma. Either genetic or chemical ablation of IRE1 resulted in reversion of tumor growth suppression under low-PROT regimen, accompanied with reduced $CD8^+$ TILs and abrogated cytokine production (summarized in **Figure 17**). In addition, retinoic acid-inducible gene-I (RIG-I) seems to participate downstream of IRE1 by triggering IFN-I type response in tumoral cells, followed by immune recognition and response. The ablation of RIG-I in cancer cells phenocopied IRE1 knock-down resistance to low-PROT diet induced immunosurveillance. Both IRE1 or RIG-I genetic deletions did not impact on tumor growth under CTR diet, indicating that in the studied model the anti-tumor properties depended on at least two elements: external stressor (low PROT regimen) and intact immunity (syngeneic mouse model). Multiple implications and questions arise from this work that need to be elucidated in the further studies.

1. Diet, metabolism and cancer

Diet is recognized as the major environmental factor affecting cancer risk and survival in humans. Unfortunately, despite the growing awareness and scientific progress in what constitutes the healthy diet worldwide a trend toward overweight and obesity is still on rise, accompanied by the prevalence of unhealthy over healthy dietary patterns (Collaboration, 2017; Imamura et al., 2015). The preventive impact of diet on cancer onset is well acknowledged and documented, in contrast to the impact of the diet on clinical outcomes in patients with diagnosed cancer. Therefore, there is a growing interest in researching the nutritional impact on already established tumors, aiming to provide additional benefits in combination with current anti-cancer therapies.

The vast majority of experimental studies investigating diet and cancer have been done in immunodeficient mouse models. The obvious limitation of such approach is the lack of immune system and its implication in tumor development, which means that the effect of the tested nutritional approach is immune system independent.

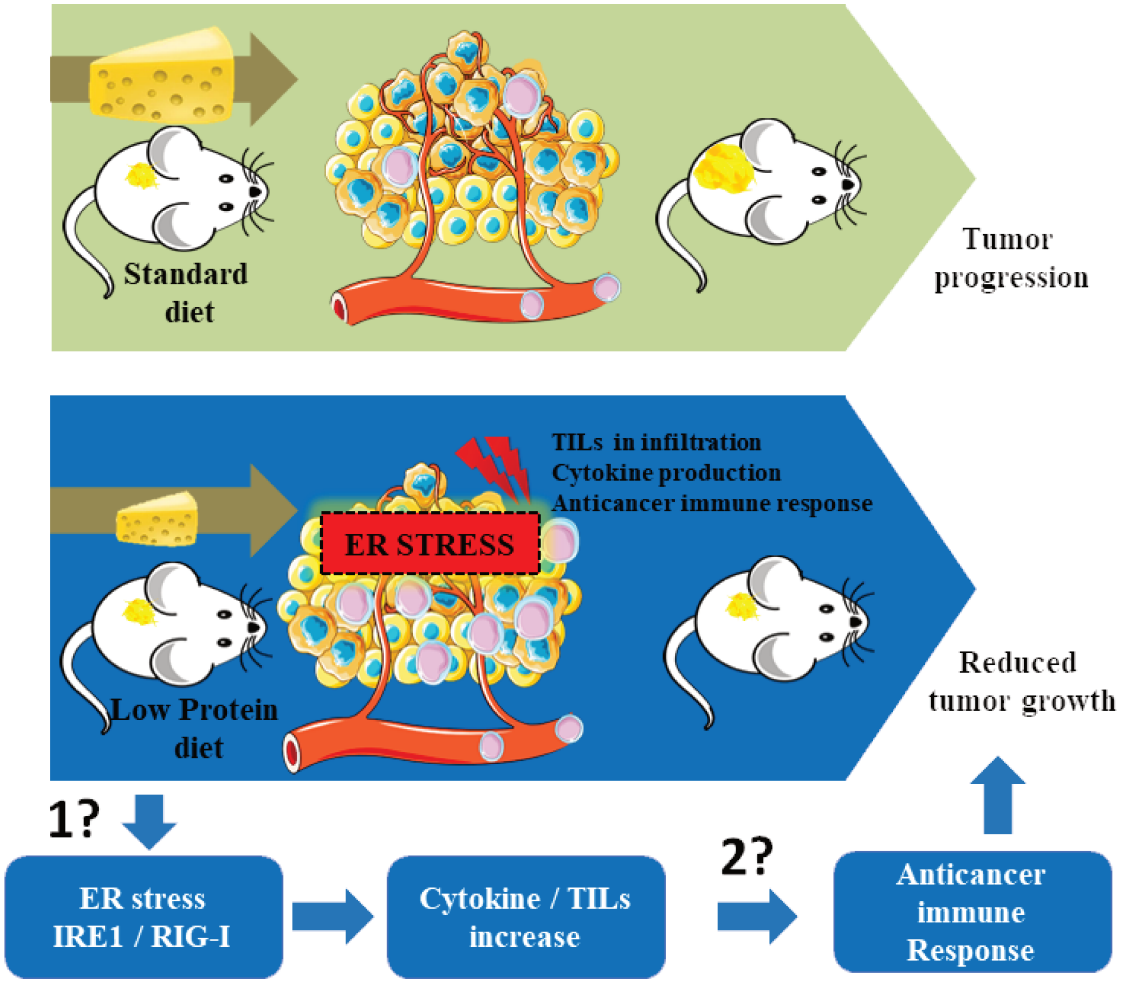


Figure 17: Low PROT induced immune response.

Syngeneic tumor bearing mice fed a diet lowered in protein content have decreased tumor progression. Low Protein diet induces activation of IRE1/RIG1 signaling within cancer cells, which favors cytokine, chemokine secretion and increased T cell tumor infiltration. This leads to enhanced anticancer immune response characterized by higher cytotoxicity towards tumoral cells. The numbers "1?" and "2?" indicates the place of the molecular links whose mechanims have yet to be determined.

Recent studies have brought attention on the importance of diet- and calorie restriction mimetic-induced anticancer immunosurveillance (Di Biase et al., 2016; Pietrocola et al., 2016). Although those researches are uncovering the importance of the immune system in anti-cancer response, they attribute this activation to the induction of autophagy within tumor cells upon mouse starvation or to enhanced protection of immune system components against anti-tumor chemotherapy by short-term-starvation. Both of these regimens impacted on mouse weight and are not easy to translate

into clinical settings. Instead, we described that mild reduction (25%) in dietary protein content only, without decreasing the overall calorie consumption and in the absence of combination with additional chemotherapy treatment, led to tumor growth reduction.

The observed reduction in tumor growth under low PROT diet regimen was entirely dependent on an effective anti-cancer immune response. This conclusion is further supported by our finding that tumor growth in an immune-deficient mouse model was accelerated under low PROT diet as

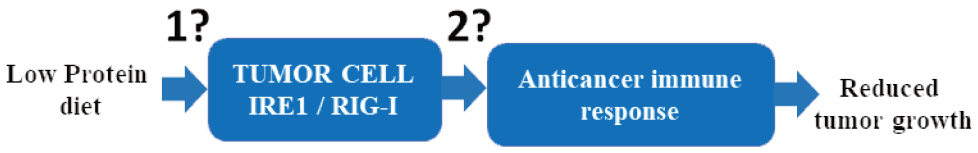


Figure 18: Recapitulative scheme of low PROT diet-induced anticancer immune response.

Low PROT diet regimen induces IRE1/RIG1 axis signaling within tumor cells. That signaling is indispensable for activation of effective anti-cancer immune response resulting in reduction in tumor growth. The two remaining open question are: "1?" how does a low PROT diet selectively activate IRE1/RIG1 signaling within tumor cells and, "2?" how is this signaling being translated into an effective anti-cancer immune response?

compared to CTR diet. We showed that this immune response is dependent on IRE1/RIG1 axis within the tumor cell population (Fig. 17). Thus, we can reason that diet is acting on tumoral cells which in turn activate an immune system response towards themselves. That scenario seems fitting to the observation that tumor growth is accelerated in the immuno-deficient mouse model under low PROT regimen, as it would be predicted from IRE1 activation which functions as the pro-survival and pro-tumoral branch of the UPR.

Establishing the above course of events (graphically represented on figures 17 and 18), leaves two unanswered questions – how can a dietary regimen result in IRE1/RIG1 signaling within cancer cells, and how is this signaling inducing an anti-cancer immune response? Some of the proposed hypothesis will be presented in this section.

Glucose and glutamine are main sources of carbon contributing to *de novo* lipid biosynthesis, but under a hypoxic environment some cancer cell lines can incorporate significant amounts of extracellular acetate to provide a precursor of acetyl-CoA and facilitate lipid biosynthesis (Kamphorst, Chung, Fan, & Rabinowitz, 2014). It is interesting to note that the work of Pietrocola et al., describes that hydroxycitrate (a calorie restriction mimetic that blocks the production of cytosolic acetyl-CoA) given chronically by itself has the ability to improve immune surveillance and reduce tumor burden. Diet-derived acetate whose significant fraction is generated by gut microbiota and contribute to generation of acetyl-CoA may be delivered by the bloodstream to supply tumor growth (Hosios & Vander Heiden, 2014). Thus, it might be possible that acetate levels could drive differential lipid metabolism under amino acid restricted conditions, or under hydroxycitrate treatment, which would drive unique stress response observed under low PROT diet conditions. In addition, the impact of the microbiota and its products (such as acetate) is another uninvestigated aspect of low PROT diet-induced anti-cancer immune response. The role of

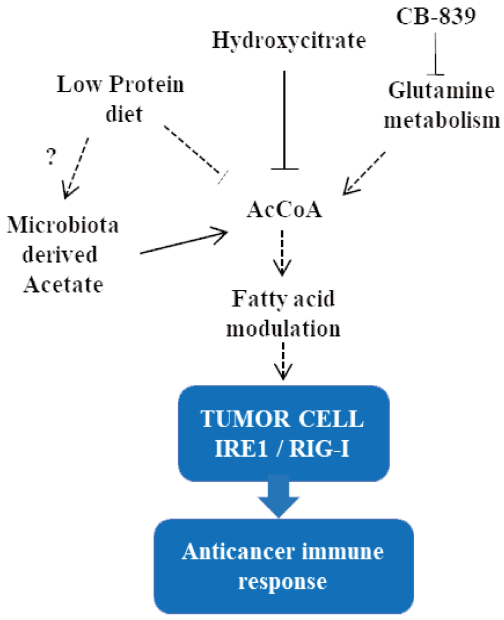


Figure 19: Open question one - Metabolic modulation that could lead to IRE1 activation.

Treatments leading to induction of anti-cancer immunosurveillance. What could be a common denominator of all of them (at least partially) is the modulation of the pool of acetyl-CoA. Ac-CoA molecules can directly impact on differential fatty acid synthesis, which in turn can lead to specific induction of IRE1 activity within tumor cells. Dashed lines indicate the hypothetical links that have yet to be tested.

microbiota and anti-cancer immune responses is one of the most debated subjects in the recent years (York, 2018; Zitvogel, Ma, Raoult, Kroemer, & Gajewski, 2018)

Acetyl-CoA – a central metabolite cross-connecting nutrient metabolism

Acetyl-CoA is mainly known as the activated carrier of the acetyl group for incorporation into the Krebs cycle to fuel mitochondrial oxidative metabolism and ATP production, but it has no less important function in lipid biosynthesis and as a donor of acetyl groups for protein acetylation. Therefore, any metabolic perturbations and stressors likely can impact or reflect in acetyl-CoA modulation. In addition, acetyl-CoA lays at the interface between central carbon and fatty acid metabolism. It has been proposed that under hypoxic conditions, for instance in case of solid tumor microenvironment, the glycolytic flux is impaired and redirected toward lactate production rather than acetyl-CoA, diminishing its availability for downstream metabolic pathways, namely fatty acid and ketone bodies synthesis, steroid biogenesis and protein acetylation. Thus, hypoxic stressed tumor cells are pushed to rely on the other acetyl precursors, mainly glutamine and acetate, but also on branched chain-amino acids and free-fatty acid extracellular absorption.

Cells can obtain required fatty acids either by absorption from the environment or by *de novo* lipid biosynthesis, and cancer cells in general prefer the second way. For that, they require constant pool of acetyl-CoA as a main precursor of fatty acid biogenesis. It has been established that a substantial fraction of cytosolic acetyl-CoA does not come from glucose or glutamine in hypoxic cells.

As mentioned, specific IRE1 activation can be the result of lipotoxicity (increase in saturated versus unsaturated fatty acid accumulation within the ER membrane), but simultaneously UPR has been implicated in modulation of lipogenesis by activation of sterol regulatory element-binding proteins (SREBP) (J. Y. Kim et al., 2018). More importantly, this UPR induction has been accompanied by increased immune cells infiltration in the mouse model of high fat diet-induced hepatitis, more specifically by macrophage infiltration (Nakagawa et al., 2014). It would be interesting to test by metabolomics whether there is a change in the lipidomic profile of tumors developed under low PROT diet to have an insight in the metabolic alterations leading to specific activation of IRE1.

GCN2 is an important sensor of amino-acid availability in the cell. Upon amino acid deprivation, the pool of non-bound tRNA rises, which induces phosphorylation of GCN2 and triggers ATF4

transcriptional activation (Ye et al., 2010). Independently, GCN2 participates in the mTOR inhibition, possibly by the regulation of eIF2α, which once it is activated halts protein translation (Averous et al., 2016). Autophagy can be also induced and in some context dependent on GCN2 activation (X. Xia et al., 2018; X. J. Xia et al., 2016). Interestingly, GCN2-ATF4 signaling under nutrient scarcity has been linked to increased flux of hexosamine biosynthetic pathway through transcriptional regulation of the step-limiting enzyme GFAT1 (Chaveroux et al., 2016). Hexosamine pathway is strongly implicated in the production of N-acetylglucosamine, substrate that is used in protein maturation to be attached to hydroxyl group of serine or threonine amino acid residues in a process termed *O*-GlcNAcylation (Harwood & Hanover, 2014). *O*-GlcNAcylation has been recognized as an important mechanism in cancer biology, development and progression, impacting tumor metabolism and possibly anticancer immune response (X. Yang & Qian, 2017).

Under our low PROT diet we have found mild but significant reduction of many amino acids within the tumor microenvironment (Article 1 Fig. S4F). Despite of this decrease, we have not found any signs of GCN2 nor eIF2α phosphorylation in the tumors of mice under low PROT diet regimen (Article 1 Fig. S4C and S5A). mTOR and AKT pathways were not modulated either (Article 1 Fig. S4A-B). In addition, autophagy was not increased as tested by LC3 lipidation (Article 1 Fig. S4D). Most importantly, ATF4 was not induced under low PROT diet (Article 1 Fig. S5A-B). Hence, the magnitude of amino acid decrease in our model does not seem to be enough to trigger GCN2 or autophagy signaling.

As we have observed differential modulation in amino acid content between tumors dissected from mice under control and Low PROT diets, with particular shift toward higher glutamine/glutamate ratio, we decided to investigate the effect of altered glutamine metabolism on the status of IRE1 activity and MHC-I expression. That glutamine/glutamate shift could be the result of differential extracellular glutamine import, but as GCN2 signaling was not affected, we reasoned that it is more likely to be the alteration in the enzymatic conversion of glutamine to glutamate. Thus, we used CB-839, a specific glutaminase inhibitor, to block glutamine to glutamate conversion, which we expected to partially mimic the phenotype we observed *in vivo*. First, we established the conditions and dose where CB-839 had minimal impact on cells viability (Preliminary results Fig. 2A). In

such an experimental set-up CB-839 treatment induced IRE1 activation, as assessed by IRE1 phosphorylation and sXBP1 levels (Preliminary results Fig. 2C and D). Interestingly, it did not induce general acute ER stress, as the extent of CHOP induction was minor as compared to glutamine deprivation (Preliminary results Fig. 2E). At the same time MHC-I surface expression was markedly induced (Preliminary results Fig. 2B), indicating that alterations in glutamine metabolism indeed can lead to modulation in cell surface MHC-I expression, simultaneously inducing IRE1 activity. Further studies will be needed to establish whether these pathways are connected at the molecular level, or whether they are independent but induced in parallel by a common trigger.

In the context of GCN2 activation in tumor progression, recent findings uncovered one way by which GCN2 promotes cancer survival and resistance upon chemotherapeutic asparaginase therapy (Nakamura et al., 2018). Interestingly from our standpoint, this study also uncovers some GCN2 independent phenotype of asparaginase treatment. As some cancers are dependent on extracellular asparagine, removal of this amino acid from the tumor environment by enzymatic degradation has been proposed as an efficient way to starve cancer cells. GCN2 activation was shown to attenuate asparaginase induced cell death via ATF4-driven stress response within wide range of cancer cell types. Interestingly, the transcriptional profiling of human leukemic lymphoblasts CCRF-CEM treated either with the asparaginase alone on in combination with a GCN2 inhibitor revealed molecular pathways that were induced by asparagine depletion independent of downstream GCN2 signaling. Upon GCN2 inhibition, upstream bioinformatic analysis indicated activation of pathways under regulation of Epidermal growth factor, IFNγ and TNFα. This indicates that amino acid deprivation in the form of asparaginase treatment induced pro-inflammatory phenotype, but in an GCN2-independent manner. Thus, it surprisingly phenocopies some of the aspects of the low PROT diet that we described as independent of GCN2 activity.

In summary, the mechanisms of how low PROT diet induce specific IRE1/RIG1 axis in tumor cells are not yet determined and can be transmitted by indirect action of various components (metabolites, microbiota, fatty acid biosynthesis or others not known). It will be the goal of future studies to establish the exact metabolic modulation within cancer cells that leads to IRE1 induction.

2. IRE1 and cell secretome

As IRE1 is central in proteostasis and stress response, it is likely that IRE activation would result in enhanced resistance of tumor cells to death and facilitate survival in the hostile environment and toxicity upon chemotherapeutic treatment. Indeed, IRE1 RNase activity ablation was shown to sensitize breast cancer tumors to paclitaxel treatment and prolong survival of triple negative breast cancer (TNBC) tumor-bearing immunodeficient mice (Logue et al., 2018). In this study Logue et al. screened a panel of human breast cancer cell lines and observed differential expression of sXBP1 protein and mRNA under basal conditions, with the highest levels in TNBC cell lines and cell lines with hormone receptor expression (estrogen and (hormonal epithelial growth factor receptor 2 (HER2) receptors). They have demonstrated that *in vitro* secretion of pro-inflammatory cytokines (CXCL1, IL6 and IL8 amongst others) is partially IRE1-dependent and promote cancer proliferation via cytokine autocrine loop. However, this enhanced proliferation effect was not reproduced *in vivo* when IRE1 RNase inhibitor was used as a single treatment. When combined with the chemotherapeutic paclitaxel, IRE1 RNase inhibition resulted in tumor growth reduction. Mechanistically the molecular observation of Logue et al. associating cytokine production dependent of IRE1 activity is in agreement with our findings, as in the case of IRE1 activation we have observed induction of pro-inflammatory cytokines in tumor cells (Preliminary results Fig. 4B). In addition, we have also observed acceleration of tumor progression in an immunodeficient mouse model (Article 1 Fig. 1 C-D), which could be explained by IRE1-dependent cytokine induction under low-PROT diet. In summary, in the context of immunocompetent mice IRE1 signaling seems to exert dominantly anti-tumor effects by dominant role of immune system activity in tumor development, at least within the tested cancer models.

As it also happens, TNBC cells in contrast to the other studied breast cancer cell lines heavily rely on extracellular glutamine levels and exhibit high expression of glutamine converting enzymes, which make them more sensitive to glutaminase inhibitors treatment (Gross et al., 2014; L. Zhu, Ploessl, Zhou, Mankoff, & Kung, 2017). As glutamine deprivation is known to induce UPR, it would be of high interest to investigate if the obtained differences in sXBP1 levels (that Logues et al. observed both in basal culture conditions and *in vivo*) are not due to differential glutamine requirements among breast cancer types.

UPR and low-grade inflammation – lesson from aging

As mentioned in the chapter of aging and UPR, aging is tightly correlated with increased proteotoxicity as a result of damaged polypeptide, protein aggregate accumulation and decreased UPR functionality. In the context of longevity and increased health-span, reducing the low-grade body inflammation seems to be one of the most accepted and described ways. Furthermore, low-grade chronic inflammation is also tightly connected with increased incidence of cancer. Hence, acute inflammation (wound healing) is positive, but prolonged inflammatory processes are detrimental, and in the context of cancer, it seems to be of the utmost importance to switch towards adaptive immunity, otherwise persistent innate inflammation would accelerate cancer progression. As the immune regulation is tightly controlled and regulated at many levels, the molecular switch between innate and adaptive immunity is likely to depend on more than a single signaling pathway or molecule. It is appealing to speculate that, at least partially, this switch could be dependent on specific tumoral cell secretome orchestrated by the UPR. More specifically, secretion of inflammatory factors might be dependent on the IRE1 activation, evolutionarily the most conserved branch and the only one existing ER stress sensor in simpler organisms.

This area of the reciprocal regulation between tumor and immune system is still under vigorous scientific investigation. Future studies will reveal to what extent the proposed engagement of UPR in shaping the character of anti-cancer immune response is true.

Altered lipid metabolism and IRE1 modulation

Solid tumors have been found to alter their lipid metabolism, partially by stimulating their *de novo* lipid biosynthesis from precursors. Lipid oxidation has been tightly connected with low-grade inflammation and stress signaling and has been shown to play a role in both innate and adaptive immune responses. As it has been discussed and shown by many studies, IRE1 can be directly induced by lipid-membrane stress and related lipotoxicity (Ariyama, Kono, Matsuda, Inoue, & Arai, 2010; Covino, Hummer, & Ernst, 2018; Halbleib et al., 2017; Kitai et al., 2013; Lancaster et al., 2018; Volmer, van der Ploeg, & Ron, 2013). Particularly, the ratio between saturated and non-saturated fatty acid seems to play central role in lipotoxicity and UPR activity. This could add another factor contributing to the UPR activation within tumor cells, as it is evident that tumor

tissue differs vastly in its lipidomic profile as compared to non-malignant tissue. We have observed that with fatty acid Palmitate *in vitro* stimulation in CT26 cancer cells IRE1 is being activated (Supplementary results Fig. 1). We have hypothesized that the observed alterations in amino-acid profile between tumors dissected from CTR and low PROT diet fed mice could result in differential fatty acid metabolism within tumor cells. The future experiments with the characterization of tumor lipidome and metabolome would bring more data to verify this hypothesis.

In turn, IRE1 can participate in *de novo* lipid bio-synthesis as its RNase activity was indispensable in oncogene-induced gene expression of fatty acid synthesis enzymes such as of HMGCR1, HMGCS1, ACLY, ACACA, FASN and SCD (Xie et al., 2018).

IRE1-RIG-I axis

As described previously, a clear link exists between the UPR and RIG-I activation. RIG-I belongs to the retinoic-acid-inducible gene-I-like receptors (RLRs), that are RNA helicases sensing pathogenic RNA and initiating antiviral immunity. Studies have linked IRE1 with the RIG-I pathway via production of cleaved endogenous non-shielded short RNAs by IRE1 RNase RIDD activity. Those RIDD products could resemble pathogenic RNA as they lack 3' poly-A tail and 5' cap, normally being present in endogenous mRNA. These fragments recognised as non-self cellular RNAs can be sensed by RIG-I that would induce innate immune response by turning on production of specific cytokines, like type I-IFNs, CXCL10 and IL-6, accompanied also by MHC class I upregulation (K. Li, Qu, Chen, Wu, & Shi, 2017). Endogenous non-shielded RNAs have already been shown to play role in cancer by acting as DAMPs (K. Li et al., 2017). In immunocompetent mice, RIG-I induces anticancer immune response against melanoma cells by stimulating the secretion of extracellular vesicles carrying NKp30-ligand (BAG6) that act as immune activators (Dassler-Plenker et al., 2016). RIG-I-dependent antitumor immune response involves myeloid and plasmacytoid DCs activation, NK cells, T lymphocytes and is strongly associated with the secretion of IFNs-I (Besch et al., 2009; Dassler-Plenker et al., 2016; Poeck et al., 2008). Interestingly, we have also observed RIG-I activation in the tumors under low-PROT diet regimen, where we could attribute it to the activation of the IRE1 branch of UPR (Article 1 Fig. 4D-E and Fig.6I).

Additional observation linking RIG-I with induction of the anti-cancer immune response come from the study where pancreatic cancer cells were treated with RIG-I activating ligands which resulted in canonical ICD induction, accompanied with CLR and HMGB1 exposure (Duewell et al., 2014). As we did not observe impairment in tumor cell viability under low PROT diet feeding that would result in ICD induction, we report novel undetermined character of RIG-I signaling in the anti-cancer immune response activation. We characterized RIG-I as downstream hub of UPR activation, it is of high importance to note the recently proposed mechanism involving RIG-I signaling as a new paradigm of innate immunity activation which can sense viral infection via the UPR. Briefly, this sensing is the result of high demand on protein synthesis by viruses that hijacks the host protein synthesis machinery to produce vast amount of structural viral proteins necessary for its replication and spreading (J. A. Smith, 2014). This proposed paradigm shares some important features linking UPR with RIG-I and subsequent immune response, that resemble some of the signaling pathway that we have described under low PROT diet condition.

In similar manner, additional data bridging viral-infected tumor phenotype with increased $CD8^+$ T cell anti-tumor response come from the study where tumor-bearing mice were infected with replication-competent vaccinia virus. Upon virus treatment, researchers have observed immune system activation and anti-tumor cytotoxicity more widespread than the actual site of infection, providing additional protection against tumor invasion and metastasis (M. Kim et al., 2018). Thus, general adaptive immune response is likely to be involved in this viral-infected model in the long-term protective anti-tumor immunity. It would be of high interest to investigate whether RIG-I deficient cells would provoke such immune response upon similar treatment.

Recently RIG-I activation has been shown to induce innate antitumor activity dependent on NK cells. In mouse melanoma model, RIG-I stimulation induced secretion of extracellular vesicles expressing on its surface NKp30-ligand Bag6 that triggered NK cells activation and anti-tumor immune response resulting in inhibition of tumor growth (Dassler-Plenker et al., 2016). Bag6 is known as a quality check point protein which participates in formation of complexes directing ubiquitinated proteins towards proteasomal degradation (ERAD). Intriguingly, Bag6 was described as part of a cluster of genes located within the MHC locus and strongly induced upon IFNγ treatment. Thus, it is possible that in addition to its immunogenic activity, Bag6 could serve also

as antigen-processing regulator (Anton & Yewdell, 2014). It will be the matter of future studies to decipher if the above described mechanisms are present and/or significant impact on differences in anti-cancer immune response between CTR and low PROT diet regimen.

3. ICD and tumor immunogenicity

The second open question arising from our studies is how IRE1 signaling facilitate anti-cancer immune response (Fig. 18 marked "2?"). That link is not necessarily intuitive, because IRE1/sXBP1 is usually (but not always) linked to cell survival, poor patient prognosis and late stages of tumor development (Avril, Vauleon, & Chevet, 2017; Sakatani et al., 2017).

Immunogenic ER-resident lectin-like chaperone calreticulin (CRT) exposure occurs through a phylogenetically conserved stress pathway depending on the chemokine CXCL8 (known as IL-8) and PERK/eIF2α activation. We have found no indication that PERK/eIF2α is activated under low PROT diet regimen. Therefore, the magnitude of ER stress within tumor is likely not sufficient to promote CRT exposure. The unaffected tumor growth under low PROT diet in $CD8^+$ T cell-depleted immunocompetent mice and in an immunodeficient mouse model also points to lack of spontaneous cell death of tumor cells under low PROT diet feeding. Mitoxantrone (MTX), one of the best studied ICD-inducer has been shown to facilitate IL-8 secretion in tumor cells *in vitro* as well as in mouse tumors *in vivo* (Sukkurwala et al., 2014). It should be noted that IRE1 silencing has been shown to strongly attenuate IL-8 gene expression and secretion by Logue et al., and in addition IRE1 activation gene signature correlates with higher IL-8 mRNA in tumor of breast cancer patients (Logue et al., 2018). Therefore, the immunogenicity of cancer cells, facilitated by CRT exposure and/or IL-8 induction, could be in some conditions at least partially dependent on IRE1 signaling. It has to be stated here that high expression levels of IL-8 and its receptor CXCR2 have been associated with poor prognosis in several type of cancer (Piperi et al., 2011; Saintigny et al., 2013). On the other hand, that signaling would facilitate the antigen uptake by APCs and the subsequent presentation of antigens to T lymphocytes, which in turn would promote an anticancer immune response (Sukkurwala et al., 2014). It is not an isolated case when the molecular pathway is being attributed with enhanced immune response, yet in retrospective patient studies turns out to be associated with poor disease outcome.

The conflicting data on allowing an effective immune response with ICD and cancer progression between experimental animal models and clinical observation/trials are a continued area of debate (Galluzzi, Buque, Kepp, Zitvogel, & Kroemer, 2017b; Hou, Greten, & Xia, 2017). It has been correctly pointed out that some ICD inducers also significantly increase pro-inflammatory and tumor growth promoting signaling molecules, such as IL-1α, IL-6 and TLR-signaling cascades. As such, instead of slowing cancer progression through immune response, a pro-inflammatory environment accelerates cancer growth and shortens patient lifespan. Moreover, neoplasms that have already evolved either high immune suppression mechanisms or low immunogenic phenotypes can exhibit very high resistance to immunomodulatory therapies. Without basal anti-cancer immunity, or in case where physiological immunity is impaired, ICD-inducing therapies could be unable to provide all the benefits expected by their actions. Nevertheless, it is an open question which percentage of cancer cases and to what extent can benefit from immune targeted therapies. Accumulating preclinical and clinical data support the notion that the long-term disease outcome is far more dependent on anti-cancer immunological memory and immunosurveillance (Emens et al., 2017; Galluzzi, Buque, Kepp, Zitvogel, & Kroemer, 2015; Papaioannou, Beniata, Vitsos, Tsitsilonis, & Samara, 2016).

The puzzling paradox emerges from studies where UPR have been reported to promote cancer progression and from evidence implicating UPR in induction of an effective anti-cancer immune response which favours ICD. The patient survival prognosis is based on the cancer phenotype after cancer detection. Once cancer is diagnosed, it is very frequent that the onset of malignant transformation had occurred months/years before detection, allowing tumoral cells escape all immune barriers elicited by the organism. Thus, UPR activation at this late stage would only make an impact as a pro-survival, anti-proteotoxic mechanism that would promote faster tumor growth and cell death escape. Hence, we can predict/speculate what conditions have to be met to trigger an effective anti-tumor UPR-driven immune response:

-functional immune system

-basal immunogenicity of cancer cells

-basal TILs infiltration

-specific UPR modulation, promoting an ICD-like phenotype

Unless **all of** the above criteria are **simultaneously** present, UPR modulation and specific IRE1 induction might not be the optimal strategy to reduce cancer progression. In fact, in that scenario, they are likely to promote tumor progression due to their pro-survival character and implication in cytokine production. It would be helpful to look at the above criteria from the perspective of clinical and experimental studies. In clinics, the first two of them – "functional immune system" and "basal immunogenicity of cancer cells" are frequently absent. In fact, the tumor growth is dependent on at least one out of this two components being dysfunctional. The experimental set-up making use of tumor transplanted into syngeneic mouse model provides many investigational benefits, but differs in some important aspects between the spontaneous cancer development as it occurs in nature, factor that has to be acknowledged as one of the limitation of such studies. Firstly, the transplanted tumor consists of mature cancer cells that already underwent premalignant and malignant cancer evolution steps. That means that the tumor-receptor organism – "naïve" mouse in that case – have missed the first steps of cancer development and is colonized by final stage cancer cells. The successful progression of this cancer has been determined in its former host and implies that either the cancer cells escaped immune recognition, or they efficiently paralyzed the immune response against them. As they have been transplanted into new syngeneic host, where the immune system is yet intact, the two scenarios that are not mutually exclusive are likely to take place: 1- the cells are immunologically non-detectable and their presence do not induce the activation of anti-cancer immunity, or 2- initially the immune system is actively removing malignant cells, but eventually immunoinhibitory signaling and/or tumor cell number prevail over anti-cancer immunity and cancer cells continue proliferating. In the second scenario, the immune system has chance to develop an effective anti-cancer response before the accumulating mass of malignant cells and their immunoinhibitory signaling paralyze the anti-cancer response. This is not what we could expect to find in the clinical settings, where anti-cancer immunity has already been compromised in a step-by-step process during malignant transformation over years. Therefore, we have to point out that the above-mentioned experimental model constitutes an important translational limitation of our findings.

In that line, important clinical observation is the correlation of sXBP1 tumor expression in patient prognosis. In tumor biopsies of patients with TNBC a specific XBP1 gene-expression signature was strongly associated with poor prognosis, progression and metastatic events (Chen et al., 2014; H. Li et al., 2015). The poor prognosis for patients with breast cancer is strongly associated with cancer immune evasion, linked to the loss of MHC-I expression. Indeed, expression levels of human leukocyte antigen class I molecules are significantly downregulated at transcriptional level in breast cancer (Liu et al., 2012; Seliger, Maeurer, & Ferrone, 2000). As the loss of MHC-I is the mechanism recognized as major contributor for tumor immune escape, and core part of MHC-I complex assembling machinery lays within ER, it is of high relevance to investigate whether ER perturbation could result in hampered MHC-I expression, and if so, that modulation would eventually shape TIL functionality and numbers. This will be further discussed below.

Similarly, in colorectal cancer the infiltration of immune cells within the TME is an important factor of clinical tumor responsiveness to immunotherapy, and varies across the colorectal cancer stages in humans, observing high $CD8^+$ T cells in stages I, II and III and depletion of $CD8^+$ T cells in stage IV. On the contrary, Tregs are depleted in stages I and II and enriched in the last stages, pointing out the importance of adjusting therapeutic interventions according to the immune system status within the TME (Angelova et al., 2015).

Another interesting observation is the epigenetic regulation of basal IRE1 expression levels mediated by Enhancer of Zeste Homolog 2 (EZH2) levels (Bujisic et al., 2017). In this article authors compared two main families of Diffuse Large B-cell Lymphoma (DLBCL): Germinal Center B-cell-like (GCB) and Activated B-cell-like (ABC). They have noticed that IRE1-XBP1 branch is downregulated in most of the GCB-DLBCL, and sXBP1 genetic induction specifically in this DLBCL sub-type decreased tumor growth in an immunodeficient mouse xenograft model. They tested the hypothesis of EZH2 as the main epigenetic regulator of IRE1 expression using a chemical inhibitor of EZH2 activity, GSK343. GSK343 modified the methylation status of IRE1 promoter, induced its transcription and restored the IRE1 protein levels. Even though the tumor suppressive effects of IRE1 overexpression have been attributed to the modulation of sXBP1, the researchers also acknowledged the possibility that other functions of IRE1, such as RIDD, could participate in the observed effect. Additionally, it is known that IRE1 expression is positively

regulated by other branches of UPR, raising the possibility of modulation in basal IRE1 levels after genetic overexpression of sXBP1 (Blazanin et al., 2017; Tsuru, Imai, Saito, & Kohno, 2016).

Many of the promising immunomodulatory therapies fail because of the negative effect on immune system itself. For instance, histone deacetylase inhibitors (HDACi) therapy could in principle provide benefits by triggering an anti-tumor response. This would be through induction of immunogenic phenotypes of tumor cells via canonical ICD-related HMGB1 release as shown in B16 mouse melanoma (Booth, Roberts, Poklepovic, Kirkwood, & Dent, 2017). Unfortunately, HDACi can also positively modulate immune check point inhibitor molecules like PD-1 ligands, which hampers the anti-cancer immune response (Booth et al., 2017; Terranova-Barberio et al., 2017; Woods et al., 2015). In that context, the combination of HDACi with immune checkpoint inhibitors has been found to result in a survival extension. However, some promising HDACi like HDAC1/2 inhibitor can elicit strong toxicity towards immune cells, especially cytotoxic T cells, which could greatly impair the anti-cancer response and beneficial outcomes (R. B. Jones et al., 2014). The interesting similarities emerge between low PROT diet regimen and HDAC inhibitor therapy, which could result in PD-L1 upregulation and anti-cancer immune response. However, low PROT diet (-25% reduction) used in our studies did not lead to any negative effects on the immune system components, while HDAC inhibitors have shown to have risks.

Phosphorylation of Retinoblastoma Protein

Retinoblastoma (RB) is a tumor suppressor protein most known as inhibitor of cell cycle by negative regulation of E2F family of proteins. RB phosphorylative inactivation or mutation during tumor progression not only stimulates proliferation by promoting the cellular G1–S transition through de-repression of E2F transcription factors, but it also impacts on a variety of other malignant events. For instance, RB has been found to modulate multiple cytokines and chemokines as well as cancer stem cell markers (Kitajima et al., 2017). Upon RB genetic invalidation in the p53-null breast cancer cell line MCF-7, the transcriptional levels of IL6, CXCL1/2/3/5 have been drastically increased, followed by increase in stem cell markers. In addition, chemokines such as CCL2 and CCL5 were markedly upregulated. On the other hand, genes involved in lipid biosynthesis and glutamine to glutamate conversion were downregulated (PPP1R3, GLS) as well as PGAM1. PGAM1 is one of the first enzyme of the glycolytic pathway whose downregulation

can disrupt the glycolytic flux and affect the oxidative branch of the pentose phosphate pathway and the serine biosynthesis pathway (Chaneton & Gottlieb, 2012). Oncogene KRAS was another target downregulated upon RB inactivation. MHC-I assembling machinery was also upregulated (TAPBP, TAP1, HLA-A/J/G and most importantly ERAP1), pointing out the possible role of RB in immune detection and response. This idea reflects a hypothesis that has been already proposed in some of the researches indicating the role of pRB in interferon-modulated pathways and MHC-II regulation (Kitajima & Takahashi, 2017; X. Zhu, Pattenden, & Bremner, 1999). Of note, expression of XBP1 and its target EDEM1 was attenuated under inactivation of RB, suggesting that this tumor suppressor may be upstream of their regulation, which would be consistent with previous reported studies (Brewer, Hendershot, Sherr, & Diehl, 1999).

In our model of low PROT diet-induced tumor growth reduction we surprisingly observed increased phosphorylation of RB as compared to tumors dissected from mice under control diet (Preliminary results Fig.5). That would imply that cell cycle rates and proliferation could be increased in conditions of low PROT diet. Nevertheless, overall tumor growth was suppressed in those conditions, in contrast to the experiment performed in immunodeficient mouse model, the case that was already discussed in chapter “IRE1 and cell secretome”. It is reasonable to hypothesize that even though cancer cells received growth-promoting signals by phosphorylation of RB, the immune system negated this effect. Would it mean that by promoting the RB activity we could expect even higher tumor growth suppression under low PROT diet regimen? Unless experimentally tested, we can neither answer, nor rule out this question, but it is plausible that inactivation of RB by phosphorylation could be necessary to induce, at least partially, an effective immune response via regulating the immunogenicity of cancer cells. It might be possible that tumor cells in contrast to healthy cells metabolically fail to adapt to low PROT diet-TME conditions. This could lead as explained by the differential stress sensitization (DSS) phenomenon to tumor cell proliferation even without necessary nutrients, which might render malignant cells prone to un-controlled death. In addition, RB is implicated in IFNγ-driven MHC class II regulation and IL-6 production (upon RB phosphorylation), which might actually promote immune activation (Jung et al., 2012; Kitajima et al., 2017; X. Zhu et al., 1999). Interestingly, NKG2D ligands such as RAE-1 (discussed below) could be also regulated through E2F transcription factors, key downstream

targets of RB (Jung et al., 2012). As such, pRB-driven E2F activity could lead to expression of immunogenic NKG2D ligands and alert immune system to eliminate malignant cells.

MHCI and MHCI-like family of proteins

As it is shown in preliminary results Fig. 4A, MHC class I (MHC-I) surface expression is significantly induced in tumor cells isolated from mice bearing CT26 colorectal carcinoma (CRC) cells under low PROT feeding. MHC-I molecules are present on almost all nucleated cells in mammals and display oligopeptides on the cell surface which are recognised by $CD8^+$ T cell receptors, enabling T cell activation in the presence of secondary signals that results in T cell immunosurveillance and cytotoxicity.

Importantly this low-PROT diet induced MHC-I expression was dependent on IRE1 (Preliminary results Fig. 1A). As cancer cells isolated from tumor bearing mice under low-PROT diet exhibited significant increase in MHC-I on their cell surface (Preliminary results Fig.4A), it would be of high interest to investigate if this phenotype is contributing to higher immune response observed in that group. In addition, we have found elevated mRNA levels of key MHC-I assembling machinery proteins such as *TAP1* and *TRIM69* (Preliminary results Fig. 4C). On the other hand, this upregulation could be the direct consequence of IFNγ signaling, which is a known potent induced of MHC-I expression.

Intriguingly, ER stress has controversial role in MHC-I processing and exposure. ER stress induction has been linked to both decrease (de Almeida, Fleming, Azevedo, Carmo-Fonseca, & de Sousa, 2007; Granados et al., 2009; Ulianich et al., 2011), and increase in MHC-I expression (Gameiro et al., 2014; Malamas, Gameiro, Knudson, & Hodge, 2016). In our *in vitro* experiments, general induction of ER stress by treatment with a chemical blocker of N-linked glycosylation (Tunicamycin) indeed led to massive downregulation in MHC-I surface expression on CT26 cells (Preliminary results Fig. 1D). However, specific IRE1 induction by palmitic-acid treatment resulted in time-dependent increase in MHC-I expression (Preliminary results Fig. 1A-D). However, those experiments have to be reproduced in IRE1-supressed cells in order to test whether this induction is being dependent on its signaling or is it an effect of another mechanism.

The other interesting modulation found on tumoral cells under low PROT diet regimen is the modulation of PD-L1. PD-L1 expression on hepatocellular carcinoma (HCC) is potently induced by IFN-γ release from tumor-infiltrating T cells *in vivo* and is not observed in cell culture conditions (Concha-Benavente et al., 2016; Sanmamed & Chen, 2014). IFN-γ is mainly released by T cells upon antigen recognition and T cell activation and serves prolonged T-cell cytotoxic response and amplification. Thus, induction of PD-L1 by IFN-γ represents an immune self-limiting activation mechanism, named "adaptive immune resistance" (Dosset et al., 2018).

The crucial importance of PD-L1/PD1 axis in inducing long-term tumor rejection has been recently shown in scientific works investigating immunostimulatory chemotherapies in combination with anti-PD1 treatment. The researchers have investigated the effect of 5-Fluorouracil (5-FU), Oxaliplatin (Ox) and Mitomycin C (MitoC) as single agents and the combination of 5-FU with Ox, named Folfox, and 5-FU plus MitoC. Whereas Ox is known as an inducer of ICD, 5-FU was shown to deplete myeloid-derived supressor cells (MDSCs), and MitoC is a non-ICD chemotherapeutic. The double treatments, Folfox and 5-FU/MitoC groups resulted in slight tumor growth retardation in CT26-BALBc mice, and no tumor rejection was observed in all groups. Only under the combination of Folfox with anti-PD1 treatment the investigators observed massive effect on mouse survival as 92% of the mice were alive at day 17 after beginning the treatment compared to 56% in Folfox alone and 0% in control group. In addition, 62% of mice underwent complete tumor regression and sustained long-term immunoprotection specific towards CT26 cancer cells in the combined therapy with Folfox and anti-PD1. More importantly, MitoC provided no additional effect combined with anti-PD1 treatment, indicating immune modulation and ICD as central events in tumor suppression and rejection. Mechanistically, Folfox treatment induced $CD8^+$ T cell intratumoral infiltration, increased numbers of IFNγ positive $CD8^+$ T cells and promoted IFNγ release in response to immunogenic peptide binding. On the other hand, this combined treatment also upregulated translational and surface expression of immune inhibitory receptors, like PD-1 and Tim-3, making T cells susceptible to exhaustion, which was indeed observed as $CD8^+$ T cells progressively lost their antitumor activity *in vivo* when not combined with anti-PD1 therapy. At the same time, in response to T cell-derived IFNγ tumor cells upregulated its PD-L1 expression, that further facilitated immune suppression. In accord with this result, we have observed significant increase in PD-L1 expression on isolated tumor cells from mice under low PROT diet regimen

(Preliminary results Fig. 4A). Even though this modulation of PD-L1 did not hampered low PROT induced immune response, it could be interesting to test whether the combination of anti-PD-L1 therapy would result in additional beneficial response when combined with low PROT diet.

Thus, IFNγ-driven induction of a variety of immunoinhibitory ligands on tumor cells renders resistance towards immunomodulatory therapies (Benci et al., 2016). It would be interesting to examine whether specific UPR activation, centred on IRE1, would somehow abrogate this immune-inhibitory potential of cancer cells, by for instance, modulation of the cell secretome and surface expression of immune checkpoint ligands.

The tumor was called “wound that never heal”. In the case of a real wound, immune cells can vastly infiltrate it and their growth can be promoted by growth factors resulting in a beneficial effect. However, this inflammatory process is detrimental in the context of cancer. As summarized in numerous studies, many cytokines have dual roles in tumor progression. The same interleukin can promote or inhibit tumor growth, depending on the context. For example TGF-β produced by tumor cells and surrounding stromal cells, facilitates cancer invasion and metastases, but in the earlier phases of tumor development it acts as a tumor suppressor, inhibiting cell growth, inducing apoptosis and attenuating growth signals such as the proto-oncogenic c-Myc (S. Lee & Margolin, 2011).

Similarly, cells producing those cytokines seem to reflect the same properties, namely suppressing or accelerating tumor growth depending on the context. Hence, γδ T cells that are an important component of TILs in patients bearing different types of cancer possess a potent antitumor activity mediated by production of proinflammatory cytokines, direct cytotoxic activity and cross-talk with other immune populations residing within TME. The importance of γδ T cells has been highlighted by analysis of ~18,000 transcriptomes from 39 human tumors, where tumor-infiltrating γδ T cells presence was identified as the most significant favorable prognostic marker (Lo Presti et al., 2018). Here again, cytokine IL-17 seems to have dualistic role in tumor progression, and γδ T cells have been identified as main contributors of this cytokine in TME. IL-17 promote TCR recognition and cytotoxic T cell response, but at the same time was found to accelerate tumor vascularization and growth in immunodeficient mice (S. Lee & Margolin, 2011). Of note, tumor cell recognition by γδ T cells is based on the natural killer receptors such as NKG2D and NKp30, which points out the

importance of surface expression of their ligands on cancer cells (Correia, Lopes, & Silva-Santos, 2013). The cross-talk between immune cell populations and their cytokine secretion is a critical aspect in tumor development and shapes effective anti-cancer responses. Nevertheless, in our model the central event and one of the early steps driving immune response is activation of IRE1/RIG-I axis in tumor cells. Thus, in the following studies the differential phenotype of cancer cells and their secretome between IRE1/RIG-I functional and deficient cells have to be addressed in order to determine novel molecular effectors playing a role in low PROT diet induced anti-cancer immune response.

Bibliography

Aaronson SA (1991) Growth factors and cancer. Science 254: 1146-53

Ackerman D, Simon MC (2014) Hypoxia, lipids, and cancer: surviving the harsh tumor microenvironment. Trends Cell Biol 24: 472-8

Afshar-Sterle S, Zotos D, Bernard NJ, Scherger AK, Rodling L, Alsop AE, Walker J, Masson F, Belz GT, Corcoran LM, O'Reilly LA, Strasser A, Smyth MJ, Johnstone R, Tarlinton DM, Nutt SL, Kallies A (2014) Fas ligand-mediated immune surveillance by T cells is essential for the control of spontaneous B cell lymphomas. Nat Med 20: 283-90

Al-Khami AA, Zheng L, Del Valle L, Hossain F, Wyczechowska D, Zabaleta J, Sanchez MD, Dean MJ, Rodriguez PC, Ochoa AC (2017) Exogenous lipid uptake induces metabolic and functional reprogramming of tumor-associated myeloid-derived suppressor cells. Oncoimmunology 6: e1344804

Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB (2008) Cancer is a preventable disease that requires major lifestyle changes. Pharm Res 25: 2097-116

Andresen L, Skovbakke SL, Persson G, Hagemann-Jensen M, Hansen KA, Jensen H, Skov S (2012) 2-deoxy D-glucose prevents cell surface expression of NKG2D ligands through inhibition of N-linked glycosylation. J Immunol 188: 1847-55

Angelova M, Charoentong P, Hackl H, Fischer ML, Snajder R, Krogsdam AM, Waldner MJ, Bindea G, Mlecnik B, Galon J, Trajanoski Z (2015) Characterization of the immunophenotypes and antigenomes of colorectal cancers reveals distinct tumor escape mechanisms and novel targets for immunotherapy. Genome Biol 16: 64

Anisimov VN, Bartke A (2013) The key role of growth hormone-insulin-IGF-1 signaling in aging and cancer. Crit Rev Oncol Hematol 87: 201-23

Anton LC, Yewdell JW (2014) Translating DRiPs: MHC class I immunosurveillance of pathogens and tumors. J Leukoc Biol 95: 551-62

Aranda F, Bloy N, Pesquet J, Petit B, Chaba K, Sauvat A, Kepp O, Khadra N, Enot D, Pfirschke C, Pittet M, Zitvogel L, Kroemer G, Senovilla L (2015) Immune-dependent antineoplastic effects of cisplatin plus pyridoxine in non-small-cell lung cancer. Oncogene 34: 3053-62

Ariyama H, Kono N, Matsuda S, Inoue T, Arai H (2010) Decrease in membrane phospholipid unsaturation induces unfolded protein response. J Biol Chem 285: 22027-35

Aune D, Navarro Rosenblatt DA, Chan DS, Vieira AR, Vieira R, Greenwood DC, Vatten LJ, Norat T (2015) Dairy products, calcium, and prostate cancer risk: a systematic review and meta-analysis of cohort studies. Am J Clin Nutr 101: 87-117

Averous J, Lambert-Langlais S, Mesclon F, Carraro V, Parry L, Jousse C, Bruhat A, Maurin AC, Pierre P, Proud CG, Fafournoux P (2016) GCN2 contributes to mTORC1 inhibition by leucine deprivation through an ATF4 independent mechanism. Sci Rep 6: 27698

Avril T, Vauleon E, Chevet E (2017) Endoplasmic reticulum stress signaling and chemotherapy resistance in solid cancers. Oncogenesis 6: e373

Baena Ruiz R, Salinas Hernandez P (2014) Diet and cancer: risk factors and epidemiological evidence. Maturitas 77: 202-8

Bandera EV, Fay SH, Giovannucci E, Leitzmann MF, Marklew R, McTiernan A, Mullee A, Romieu I, Thune I, Uauy R, Wiseman MJ, World Cancer Research Fund International Continuous Update Project P (2016) The use and interpretation of anthropometric measures in cancer epidemiology: A perspective from the world cancer research fund international continuous update project. Int J Cancer 139: 2391-7

Bauersfeld SP, Kessler CS, Wischnewsky M, Jaensch A, Steckhan N, Stange R, Kunz B, Bruckner B, Sehouli J, Michalsen A (2018) The effects of short-term fasting on quality of life and tolerance to chemotherapy in patients with breast and ovarian cancer: a randomized cross-over pilot study. BMC Cancer 18: 476

Baumeister P, Luo S, Skarnes WC, Sui G, Seto E, Shi Y, Lee AS (2005) Endoplasmic reticulum stress induction of the Grp78/BiP promoter: activating mechanisms mediated by YY1 and its interactive chromatin modifiers. Mol Cell Biol 25: 4529-40

Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T, Tailor P, Morimoto RI, Cohen GM, Green DR (2000) Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. Nat Cell Biol 2: 469-75

Ben-Zvi A, Miller EA, Morimoto RI (2009) Collapse of proteostasis represents an early molecular event in Caenorhabditis elegans aging. Proc Natl Acad Sci U S A 106: 14914-9

Ben Sahra I, Regazzetti C, Robert G, Laurent K, Le Marchand-Brustel Y, Auberger P, Tanti JF, Giorgetti-Peraldi S, Bost F (2011) Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. Cancer Res 71: 4366-72

Benci JL, Xu B, Qiu Y, Wu TJ, Dada H, Twyman-Saint Victor C, Cucolo L, Lee DSM, Pauken KE, Huang AC, Gangadhar TC, Amaravadi RK, Schuchter LM, Feldman MD, Ishwaran H, Vonderheide RH, Maity A, Wherry EJ, Minn AJ (2016) Tumor Interferon Signaling Regulates a Multigenic Resistance Program to Immune Checkpoint Blockade. Cell 167: 1540-1554 e12

Beneteau M, Zunino B, Jacquin MA, Meynet O, Chiche J, Pradelli LA, Marchetti S, Cornille A, Carles M, Ricci JE (2012) Combination of glycolysis inhibition with chemotherapy results in an antitumor immune response. Proc Natl Acad Sci U S A 109: 20071-6

Besch R, Poeck H, Hohenauer T, Senft D, Hacker G, Berking C, Hornung V, Endres S, Ruzicka T, Rothenfusser S, Hartmann G (2009) Proapoptotic signaling induced by RIG-I and MDA-5 results in type I interferon-independent apoptosis in human melanoma cells. J Clin Invest 119: 2399-411

Biancur DE, Paulo JA, Malachowska B, Quiles Del Rey M, Sousa CM, Wang X, Sohn ASW, Chu GC, Gygi SP, Harper JW, Fendler W, Mancias JD, Kimmelman AC (2017) Compensatory metabolic networks in pancreatic cancers upon perturbation of glutamine metabolism. Nat Commun 8: 15965

Blazanin N, Son J, Craig-Lucas AB, John CL, Breech KJ, Podolsky MA, Glick AB (2017) ER stress and distinct outputs of the IRE1alpha RNase control proliferation and senescence in response to oncogenic Ras. Proc Natl Acad Sci U S A 114: 9900-9905

Blees A, Reichel K, Trowitzsch S, Fisette O, Bock C, Abele R, Hummer G, Schafer LV, Tampe R (2015) Assembly of the MHC I peptide-loading complex determined by a conserved ionic lock-switch. Sci Rep 5: 17341

Blot WJ, Tarone RE (2015) Doll and Peto's quantitative estimates of cancer risks: holding generally true for 35 years. J Natl Cancer Inst 107

Bonorden MJ, Rogozina OP, Kluczny CM, Grossmann ME, Grambsch PL, Grande JP, Perkins S, Lokshin A, Cleary MP (2009) Intermittent calorie restriction delays prostate tumor detection and increases survival time in TRAMP mice. Nutr Cancer 61: 265-75

Booth L, Roberts JL, Poklepovic A, Kirkwood J, Dent P (2017) HDAC inhibitors enhance the immunotherapy response of melanoma cells. Oncotarget 8: 83155-83170

Bordone L, Cohen D, Robinson A, Motta MC, van Veen E, Czopik A, Steele AD, Crowe H, Marmor S, Luo J, Gu W, Guarente L (2007) SIRT1 transgenic mice show phenotypes resembling calorie restriction. Aging Cell 6: 759-67

Boutant M, Kulkarni SS, Joffraud M, Raymond F, Metairon S, Descombes P, Canto C (2016) SIRT1 Gain of Function Does Not Mimic or Enhance the Adaptations to Intermittent Fasting. Cell Rep 14: 2068-2075

Brewer JW, Hendershot LM, Sherr CJ, Diehl JA (1999) Mammalian unfolded protein response inhibits cyclin D1 translation and cell-cycle progression. Proc Natl Acad Sci U S A 96: 8505-10

Bristow RG, Hill RP (2008) Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. Nat Rev Cancer 8: 180-92

Bruey JM, Ducasse C, Bonniaud P, Ravagnan L, Susin SA, Diaz-Latoud C, Gurbuxani S, Arrigo AP, Kroemer G, Solary E, Garrido C (2000) Hsp27 negatively regulates cell death by interacting with cytochrome c. Nat Cell Biol 2: 645-52

Bujisic B, De Gassart A, Tallant R, Demaria O, Zaffalon L, Chelbi S, Gilliet M, Bertoni F, Martinon F (2017) Impairment of both IRE1 expression and XBP1 activation is a hallmark of GCB DLBCL and contributes to tumor growth. Blood 129: 2420-2428

Buono R, Longo VD (2018) Starvation, Stress Resistance, and Cancer. Trends Endocrinol Metab 29: 271-280

Burnette BC, Liang H, Lee Y, Chlewicki L, Khodarev NN, Weichselbaum RR, Fu YX, Auh SL (2011) The efficacy of radiotherapy relies upon induction of type i interferon-dependent innate and adaptive immunity. Cancer Res 71: 2488-96

Campbell TC (2017) The Past, Present, and Future of Nutrition and Cancer: Part 1-Was A Nutritional Association Acknowledged a Century Ago? Nutr Cancer 69: 811-817

Cannarile MA, Weisser M, Jacob W, Jegg AM, Ries CH, Ruttinger D (2017) Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. J Immunother Cancer 5: 53

Cao H, Wang G, Meng L, Shen H, Feng Z, Liu Q, Du J (2012) Association between circulating levels of IGF-1 and IGFBP-3 and lung cancer risk: a meta-analysis. PLoS One 7: e49884

Carpio MA, Michaud M, Zhou W, Fisher JK, Walensky LD, Katz SG (2015) BCL-2 family member BOK promotes apoptosis in response to endoplasmic reticulum stress. Proc Natl Acad Sci U S A 112: 7201-6

Cha JH, Yang WH, Xia W, Wei Y, Chan LC, Lim SO, Li CW, Kim T, Chang SS, Lee HH, Hsu JL, Wang HL, Kuo CW, Chang WC, Hadad S, Purdie CA, McCoy AM, Cai S, Tu Y, Litton JK et al. (2018) Metformin Promotes Antitumor Immunity via Endoplasmic-Reticulum-Associated Degradation of PD-L1. Mol Cell 71: 606-620 e7

Chalkiadaki A, Guarente L (2015) The multifaceted functions of sirtuins in cancer. Nat Rev Cancer 15: 608-24

Chaneton B, Gottlieb E (2012) PGAMgnam style: a glycolytic switch controls biosynthesis. Cancer Cell 22: 565-6

Chang CL, Hsu YT, Wu CC, Yang YC, Wang C, Wu TC, Hung CF (2012) Immune mechanism of the antitumor effects generated by bortezomib. J Immunol 189: 3209-20

Chaveroux C, Sarcinelli C, Barbet V, Belfeki S, Barthelaix A, Ferraro-Peyret C, Lebecque S, Renno T, Bruhat A, Fafournoux P, Manie SN (2016) Nutrient shortage triggers the hexosamine biosynthetic pathway via the GCN2-ATF4 signalling pathway. Sci Rep 6: 27278

Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, Lim E, Tam WL, Ni M, Chen Y, Mai J, Shen H, Hu DZ, Adoro S, Hu B, Song M, Tan C, Landis MD, Ferrari M, Shin SJ et al. (2014) XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. Nature 508: 103-107

Cheong JH, Park ES, Liang J, Dennison JB, Tsavachidou D, Nguyen-Charles C, Wa Cheng K, Hall H, Zhang D, Lu Y, Ravoori M, Kundra V, Ajani J, Lee JS, Ki Hong W, Mills GB (2011) Dual inhibition of tumor energy pathway by 2-deoxyglucose and metformin is effective against a broad spectrum of preclinical cancer models. Mol Cancer Ther 10: 2350-62

Cho JA, Lee AH, Platzer B, Cross BC, Gardner BM, De Luca H, Luong P, Harding HP, Glimcher LH, Walter P, Fiebiger E, Ron D, Kagan JC, Lencer WI (2013) The unfolded protein response element IRE1alpha senses bacterial proteins invading the ER to activate RIG-I and innate immune signaling. Cell Host Microbe 13: 558-69

Choi KM, Kwon YY, Lee CK (2013) Characterization of global gene expression during assurance of lifespan extension by caloric restriction in budding yeast. Exp Gerontol 48: 1455-68

Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de Cabo R, Sinclair DA (2004) Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 305: 390-2

Colditz GA, Sellers TA, Trapido E (2006) Epidemiology - identifying the causes and preventability of cancer? Nat Rev Cancer 6: 75-83

Colditz GA, Wolin KY, Gehlert S (2012) Applying what we know to accelerate cancer prevention. Sci Transl Med 4: 127rv4

Collaboration NCDRF (2017) Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet 390: 2627-2642

Concha-Benavente F, Srivastava RM, Trivedi S, Lei Y, Chandran U, Seethala RR, Freeman GJ, Ferris RL (2016) Identification of the Cell-Intrinsic and -Extrinsic Pathways Downstream of EGFR and IFNgamma That Induce PD-L1 Expression in Head and Neck Cancer. Cancer Res 76: 1031-43

Condamine T, Dominguez GA, Youn JI, Kossenkov AV, Mony S, Alicea-Torres K, Tcyganov E, Hashimoto A, Nefedova Y, Lin C, Partlova S, Garfall A, Vogl DT, Xu X, Knight SC, Malietzis G, Lee GH, Eruslanov E, Albelda SM, Wang X et al. (2016) Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. Sci Immunol 1

Condeelis J, Pollard JW (2006) Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 124: 263-6

Cook KL, Soto-Pantoja DR, Clarke PA, Cruz MI, Zwart A, Warri A, Hilakivi-Clarke L, Roberts DD, Clarke R (2016) Endoplasmic Reticulum Stress Protein GRP78 Modulates Lipid Metabolism to Control Drug Sensitivity and Antitumor Immunity in Breast Cancer. Cancer Res 76: 5657-5670

Correia DV, Lopes A, Silva-Santos B (2013) Tumor cell recognition by gammadelta T lymphocytes: T-cell receptor vs. NK-cell receptors. Oncoimmunology 2: e22892

Cotter TG (2009) Apoptosis and cancer: the genesis of a research field. Nat Rev Cancer 9: 501-7

Covino R, Hummer G, Ernst R (2018) Integrated Functions of Membrane Property Sensors and a Hidden Side of the Unfolded Protein Response. Mol Cell 71: 458-467

Crowe FL, Key TJ, Allen NE, Appleby PN, Roddam A, Overvad K, Gronbaek H, Tjonneland A, Halkjaer J, Dossus L, Boeing H, Kroger J, Trichopoulou A, Dilis V, Trichopoulos D, Boutron-Ruault MC, De Lauzon B, Clavel-Chapelon F, Palli D, Berrino F et al. (2009) The association between diet and serum concentrations of IGF-I, IGFBP-1, IGFBP-2, and IGFBP-3 in the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev 18: 1333-40

Cubillos-Ruiz JR, Bettigole SE, Glimcher LH (2017) Tumorigenic and Immunosuppressive Effects of Endoplasmic Reticulum Stress in Cancer. Cell 168: 692-706

Cubillos-Ruiz JR, Silberman PC, Rutkowski MR, Chopra S, Perales-Puchalt A, Song M, Zhang S, Bettigole SE, Gupta D, Holcomb K, Ellenson LH, Caputo T, Lee AH, Conejo-Garcia JR, Glimcher LH (2015) ER Stress Sensor XBP1 Controls Anti-tumor Immunity by Disrupting Dendritic Cell Homeostasis. Cell 161: 1527-38

Cui HJ, Liu XG, McCormick M, Wasko BM, Zhao W, He X, Yuan Y, Fang BX, Sun XR, Kennedy BK, Suh Y, Zhou ZJ, Kaeberlein M, Feng WL (2015) PMT1 deficiency enhances basal UPR activity and extends replicative lifespan of Saccharomyces cerevisiae. Age (Dordr) 37: 9788

Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, Bartsch G, Klocker H (1994) Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res 54: 5474-8

Currie E, Schulze A, Zechner R, Walther TC, Farese RV, Jr. (2013) Cellular fatty acid metabolism and cancer. Cell Metab 18: 153-61

Curry NL, Mino-Kenudson M, Oliver TG, Yilmaz OH, Yilmaz VO, Moon JY, Jacks T, Sabatini DM, Kalaany NY (2013) Pten-null tumors cohabiting the same lung display differential AKT activation and sensitivity to dietary restriction. Cancer Discov 3: 908-21

D'Aronzo M, Vinciguerra M, Mazza T, Panebianco C, Saracino C, Pereira SP, Graziano P, Pazienza V (2015) Fasting cycles potentiate the efficacy of gemcitabine treatment in in vitro and in vivo pancreatic cancer models. Oncotarget 6: 18545-57

Dadey DYA, Kapoor V, Hoye K, Khudanyan A, Collins A, Thotala D, Hallahan DE (2017) Antibody Targeting GRP78 Enhances the Efficacy of Radiation Therapy in Human Glioblastoma and Non-Small Cell Lung Cancer Cell Lines and Tumor Models. Clin Cancer Res 23: 2556-2564

Darling NJ, Cook SJ (2014) The role of MAPK signalling pathways in the response to endoplasmic reticulum stress. Biochim Biophys Acta 1843: 2150-63

Dassler-Plenker J, Reiners KS, van den Boorn JG, Hansen HP, Putschli B, Barnert S, Schuberth-Wagner C, Schubert R, Tuting T, Hallek M, Schlee M, Hartmann G, Pogge von Strandmann E, Coch C (2016) RIG-I activation induces the release of extracellular vesicles with antitumor activity. Oncoimmunology 5: e1219827

Daud AI, Loo K, Pauli ML, Sanchez-Rodriguez R, Sandoval PM, Taravati K, Tsai K, Nosrati A, Nardo L, Alvarado MD, Algazi AP, Pampaloni MH, Lobach IV, Hwang J, Pierce RH, Gratz IK, Krummel MF, Rosenblum MD (2016) Tumor immune profiling predicts response to anti-PD-1 therapy in human melanoma. J Clin Invest 126: 3447-52

Davidson SM, Papagiannakopoulos T, Olenchock BA, Heyman JE, Keibler MA, Luengo A, Bauer MR, Jha AK, O'Brien JP, Pierce KA, Gui DY, Sullivan LB, Wasylenko TM, Subbaraj L, Chin CR, Stephanopolous G, Mott BT, Jacks T, Clish CB, Vander Heiden MG (2016) Environment Impacts the Metabolic Dependencies of Ras-Driven Non-Small Cell Lung Cancer. Cell Metab 23: 517-28

de Almeida SF, Fleming JV, Azevedo JE, Carmo-Fonseca M, de Sousa M (2007) Stimulation of an unfolded protein response impairs MHC class I expression. J Immunol 178: 3612-9

de Groot S, Vreeswijk MP, Welters MJ, Gravesteijn G, Boei JJ, Jochems A, Houtsma D, Putter H, van der Hoeven JJ, Nortier JW, Pijl H, Kroep JR (2015) The effects of short-term fasting on tolerance to (neo) adjuvant chemotherapy in HER2-negative breast cancer patients: a randomized pilot study. BMC Cancer 15: 652

Dejeans N, Barroso K, Fernandez-Zapico ME, Samali A, Chevet E (2015) Novel roles of the unfolded protein response in the control of tumor development and aggressiveness. Semin Cancer Biol 33: 67-73

Dejeans N, Manie S, Hetz C, Bard F, Hupp T, Agostinis P, Samali A, Chevet E (2014) Addicted to secrete - novel concepts and targets in cancer therapy. Trends Mol Med 20: 242-50

Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM (2005) Statins and cancer prevention. Nat Rev Cancer 5: 930-42

Dey S, Sayers CM, Verginadis, II, Lehman SL, Cheng Y, Cerniglia GJ, Tuttle SW, Feldman MD, Zhang PJ, Fuchs SY, Diehl JA, Koumenis C (2015) ATF4-dependent induction of heme oxygenase 1 prevents anoikis and promotes metastasis. J Clin Invest 125: 2592-608

Di Biase S, Lee C, Brandhorst S, Manes B, Buono R, Cheng CW, Cacciottolo M, Martin-Montalvo A, de Cabo R, Wei M, Morgan TE, Longo VD (2016) Fasting-Mimicking Diet Reduces HO-1 to Promote T Cell-Mediated Tumor Cytotoxicity. Cancer Cell 30: 136-146

Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, Archambault JM, Lee H, Arthur CD, White JM, Kalinke U, Murphy KM, Schreiber RD (2011) Type I interferon is selectively required by dendritic cells for immune rejection of tumors. J Exp Med 208: 1989-2003

Dirx MJ, van den Brandt PA, Goldbohm RA, Lumey LH (2003) Energy restriction early in life and colon carcinoma risk: results of The Netherlands Cohort Study after 7.3 years of follow-up. Cancer 97: 46-55

Doll R, Peto R (1981) The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 66: 1191-308

Dosset M, Vargas TR, Lagrange A, Boidot R, Vegran F, Roussey A, Chalmin F, Dondaine L, Paul C, Lauret Marie-Joseph E, Martin F, Ryffel B, Borg C, Adotevi O, Ghiringhelli F, Apetoh L (2018) PD-1/PD-L1 pathway: an adaptive immune resistance mechanism to immunogenic chemotherapy in colorectal cancer. Oncoimmunology 7: e1433981

Druesne-Pecollo N, Latino-Martel P, Norat T, Barrandon E, Bertrais S, Galan P, Hercberg S (2010) Beta-carotene supplementation and cancer risk: a systematic review and metaanalysis of randomized controlled trials. Int J Cancer 127: 172-84

Duewell P, Steger A, Lohr H, Bourhis H, Hoelz H, Kirchleitner SV, Stieg MR, Grassmann S, Kobold S, Siveke JT, Endres S, Schnurr M (2014) RIG-I-like helicases induce immunogenic cell death of pancreatic cancer cells and sensitize tumors toward killing by CD8(+) T cells. Cell Death Differ 21: 1825-37

Dumbrava MD, Rothschild BM, Weishampel DB, Csiki-Sava Z, Andrei RA, Acheson KA, Codrea VA (2016) A dinosaurian facial deformity and the first occurrence of ameloblastoma in the fossil record. Sci Rep 6: 29271

Eckard SC, Rice GI, Fabre A, Badens C, Gray EE, Hartley JL, Crow YJ, Stetson DB (2014) The SKIV2L RNA exosome limits activation of the RIG-I-like receptors. Nat Immunol 15: 839-45

Edinger AL (2005) Growth factors regulate cell survival by controlling nutrient transporter expression. Biochem Soc Trans 33: 225-7

Edinger AL, Thompson CB (2002) Akt maintains cell size and survival by increasing mTOR-dependent nutrient uptake. Mol Biol Cell 13: 2276-88

Eikawa S, Nishida M, Mizukami S, Yamazaki C, Nakayama E, Udono H (2015) Immune-mediated antitumor effect by type 2 diabetes drug, metformin. Proc Natl Acad Sci U S A 112: 1809-14

Eisenberg T, Knauer H, Schauer A, Buttner S, Ruckenstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, Fussi H, Deszcz L, Hartl R, Schraml E, Criollo A, Megalou E, Weiskopf D, Laun P, Heeren G, Breitenbach M et al. (2009) Induction of autophagy by spermidine promotes longevity. Nat Cell Biol 11: 1305-14

Emens LA, Ascierto PA, Darcy PK, Demaria S, Eggermont AMM, Redmond WL, Seliger B, Marincola FM (2017) Cancer immunotherapy: Opportunities and challenges in the rapidly evolving clinical landscape. Eur J Cancer 81: 116-129

Emmanuel N, Ragunathan S, Shan Q, Wang F, Giannakou A, Huser N, Jin G, Myers J, Abraham RT, Unsal-Kacmaz K (2017) Purine Nucleotide Availability Regulates mTORC1 Activity through the Rheb GTPase. Cell Rep 19: 2665-2680

Endogenous H, Breast Cancer Collaborative G, Key TJ, Appleby PN, Reeves GK, Roddam AW (2010) Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol 11: 530-42

Fang Y, Westbrook R, Hill C, Boparai RK, Arum O, Spong A, Wang F, Javors MA, Chen J, Sun LY, Bartke A (2013) Duration of rapamycin treatment has differential effects on metabolism in mice. Cell Metab 17: 456-62

Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, Dupuy F, Chambers C, Fuerth BJ, Viollet B, Mamer OA, Avizonis D, DeBerardinis RJ, Siegel PM, Jones RG (2013) AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo. Cell Metab 17: 113-24

Feng YX, Sokol ES, Del Vecchio CA, Sanduja S, Claessen JH, Proia TA, Jin DX, Reinhardt F, Ploegh HL, Wang Q, Gupta PB (2014) Epithelial-to-mesenchymal transition activates PERK-eIF2alpha and sensitizes cells to endoplasmic reticulum stress. Cancer Discov 4: 702-15

Firestein R, Blander G, Michan S, Oberdoerffer P, Ogino S, Campbell J, Bhimavarapu A, Luikenhuis S, de Cabo R, Fuchs C, Hahn WC, Guarente LP, Sinclair DA (2008) The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. PLoS One 3: e2020

Fontana L, Adelaiye RM, Rastelli AL, Miles KM, Ciamporcero E, Longo VD, Nguyen H, Vessella R, Pili R (2013) Dietary protein restriction inhibits tumor growth in human xenograft models. Oncotarget 4: 2451-61

Fritz V, Benfodda Z, Rodier G, Henriquet C, Iborra F, Avances C, Allory Y, de la Taille A, Culine S, Blancou H, Cristol JP, Michel F, Sardet C, Fajas L (2010) Abrogation of de novo lipogenesis by stearoyl-CoA desaturase 1 inhibition interferes with oncogenic signaling and blocks prostate cancer progression in mice. Mol Cancer Ther 9: 1740-54

Fuertes MB, Kacha AK, Kline J, Woo SR, Kranz DM, Murphy KM, Gajewski TF (2011) Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. J Exp Med 208: 2005-16

Fulda S (2010) Evasion of apoptosis as a cellular stress response in cancer. Int J Cell Biol 2010: 370835

Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G (2015) Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents. Cancer Cell 28: 690-714

Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G (2017a) Immunogenic cell death in cancer and infectious disease. Nat Rev Immunol 17: 97-111

Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G (2017b) Reply: Immunosuppressive cell death in cancer. Nat Rev Immunol 17: 402

Galluzzi L, Pietrocola F, Bravo-San Pedro JM, Amaravadi RK, Baehrecke EH, Cecconi F, Codogno P, Debnath J, Gewirtz DA, Karantza V, Kimmelman A, Kumar S, Levine B, Maiuri MC, Martin SJ, Penninger J, Piacentini M, Rubinsztein DC, Simon HU, Simonsen A et al. (2015) Autophagy in malignant transformation and cancer progression. EMBO J 34: 856-80

Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, Alnemri ES, Altucci L, Amelio I, Andrews DW, Annicchiarico-Petruzzelli M, Antonov AV, Arama E, Baehrecke EH, Barlev NA, Bazan NG, Bernassola F, Bertrand MJM, Bianchi K, Blagosklonny MV et al. (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ 25: 486-541

Gameiro SR, Jammeh ML, Wattenberg MM, Tsang KY, Ferrone S, Hodge JW (2014) Radiation-induced immunogenic modulation of tumor enhances antigen processing and calreticulin exposure, resulting in enhanced T-cell killing. Oncotarget 5: 403-16

Garg AD, Agostinis P (2014) ER stress, autophagy and immunogenic cell death in photodynamic therapy-induced anti-cancer immune responses. Photochem Photobiol Sci 13: 474-87

Garg AD, Maes H, van Vliet AR, Agostinis P (2015) Targeting the hallmarks of cancer with therapy-induced endoplasmic reticulum (ER) stress. Mol Cell Oncol 2: e975089

Garrido F, Aptsiauri N, Doorduijn EM, Garcia Lora AM, van Hall T (2016) The urgent need to recover MHC class I in cancers for effective immunotherapy. Curr Opin Immunol 39: 44-51

Gavilan MP, Pintado C, Gavilan E, Jimenez S, Rios RM, Vitorica J, Castano A, Ruano D (2009) Dysfunction of the unfolded protein response increases neurodegeneration in aged rat hippocampus following proteasome inhibition. Aging Cell 8: 654-65

Ghosh AP, Klocke BJ, Ballestas ME, Roth KA (2012) CHOP potentially co-operates with FOXO3a in neuronal cells to regulate PUMA and BIM expression in response to ER stress. PLoS One 7: e39586

Gomez-Bougie P, Halliez M, Moreau P, Pellat-Deceunynck C, Amiot M (2016) Repression of Mcl-1 and disruption of the Mcl-1/Bak interaction in myeloma cells couple ER stress to mitochondrial apoptosis. Cancer Lett 383: 204-211

Gonzalez CA, Riboli E (2010) Diet and cancer prevention: Contributions from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Eur J Cancer 46: 2555-62

Goubau D, Deddouche S, Reis e Sousa C (2013) Cytosolic sensing of viruses. Immunity 38: 855-69

Granados DP, Tanguay PL, Hardy MP, Caron E, de Verteuil D, Meloche S, Perreault C (2009) ER stress affects processing of MHC class I-associated peptides. BMC Immunol 10: 10

Greaves M, Maley CC (2012) Clonal evolution in cancer. Nature 481: 306-13

Green DR, Galluzzi L, Kroemer G (2014) Cell biology. Metabolic control of cell death. Science 345: 1250256

Gronich N, Rennert G (2013) Beyond aspirin-cancer prevention with statins, metformin and bisphosphonates. Nat Rev Clin Oncol 10: 625-42

Grootjans J, Kaser A, Kaufman RJ, Blumberg RS (2016) The unfolded protein response in immunity and inflammation. Nat Rev Immunol 16: 469-84

Gross MI, Demo SD, Dennison JB, Chen L, Chernov-Rogan T, Goyal B, Janes JR, Laidig GJ, Lewis ER, Li J, Mackinnon AL, Parlati F, Rodriguez ML, Shwonek PJ, Sjogren EB, Stanton TF, Wang T, Yang J, Zhao F, Bennett MK (2014) Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. Mol Cancer Ther 13: 890-901

Guarente L (2013) Calorie restriction and sirtuins revisited. Genes Dev 27: 2072-85

Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, Wei M, Madia F, Cheng CW, Hwang D, Martin-Montalvo A, Saavedra J, Ingles S, de Cabo R, Cohen P, Longo VD (2011) Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. Sci Transl Med 3: 70ra13

Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell 30: 214-26

Halama A, Kulinski M, Dib SS, Zaghlool SB, Siveen KS, Iskandarani A, Zierer J, Prabhu KS, Satheesh NJ, Bhagwat AM, Uddin S, Kastenmuller G, Elemento O, Gross SS, Suhre K (2018) Accelerated lipid catabolism and autophagy are cancer survival mechanisms under inhibited glutaminolysis. Cancer Lett 430: 133-147

Halbleib K, Pesek K, Covino R, Hofbauer HF, Wunnicke D, Hanelt I, Hummer G, Ernst R (2017) Activation of the Unfolded Protein Response by Lipid Bilayer Stress. Mol Cell 67: 673-684 e8

Hall DT, Griss T, Ma JF, Sanchez BJ, Sadek J, Tremblay AMK, Mubaid S, Omer A, Ford RJ, Bedard N, Pause A, Wing SS, Di Marco S, Steinberg GR, Jones RG, Gallouzi IE (2018) The AMPK agonist 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), but not metformin, prevents inflammation-associated cachectic muscle wasting. EMBO Mol Med 10

Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC (1990) Linkage of early-onset familial breast cancer to chromosome 17q21. Science 250: 1684-9

Han D, Lerner AG, Vande Walle L, Upton JP, Xu W, Hagen A, Backes BJ, Oakes SA, Papa FR (2009) IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell 138: 562-75

Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100: 57-70

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646-74

Handschin C (2016) Caloric restriction and exercise "mimetics": Ready for prime time? Pharmacol Res 103: 158-66

Hardie DG (2007) AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat Rev Mol Cell Biol 8: 774-85

Hart LS, Cunningham JT, Datta T, Dey S, Tameire F, Lehman SL, Qiu B, Zhang H, Cerniglia G, Bi M, Li Y, Gao Y, Liu H, Li C, Maity A, Thomas-Tikhonenko A, Perl AE, Koong A, Fuchs SY, Diehl JA et al. (2012) ER stress-mediated autophagy promotes Myc-dependent transformation and tumor growth. J Clin Invest 122: 4621-34

Harvey AE, Lashinger LM, Otto G, Nunez NP, Hursting SD (2013) Decreased systemic IGF-1 in response to calorie restriction modulates murine tumor cell growth, nuclear factor-kappaB activation, and inflammation-related gene expression. Mol Carcinog 52: 997-1006

Harwood KR, Hanover JA (2014) Nutrient-driven O-GlcNAc cycling - think globally but act locally. J Cell Sci 127: 1857-67

Heitman J, Movva NR, Hall MN (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science 253: 905-9

Henis-Korenblit S, Zhang P, Hansen M, McCormick M, Lee SJ, Cary M, Kenyon C (2010) Insulin/IGF-1 signaling mutants reprogram ER stress response regulators to promote longevity. Proc Natl Acad Sci U S A 107: 9730-5

Herranz D, Iglesias G, Munoz-Martin M, Serrano M (2011) Limited role of Sirt1 in cancer protection by dietary restriction. Cell Cycle 10: 2215-7

Herranz D, Munoz-Martin M, Canamero M, Mulero F, Martinez-Pastor B, Fernandez-Capetillo O, Serrano M (2010) Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. Nat Commun 1: 3

Hetz C, Chevet E, Harding HP (2013) Targeting the unfolded protein response in disease. Nat Rev Drug Discov 12: 703-19

Hetz C, Chevet E, Oakes SA (2015) Proteostasis control by the unfolded protein response. Nat Cell Biol 17: 829-38

Horn H, Bohme B, Dietrich L, Koch M (2018) Endocannabinoids in Body Weight Control. Pharmaceuticals (Basel) 11

Horton BL, Gajewski TF (2018) Back from the dead: TIL apoptosis in cancer immune evasion. Br J Cancer 118: 309-311

Horton BL, Williams JB, Cabanov A, Spranger S, Gajewski TF (2018) Intratumoral CD8(+) T-cell Apoptosis Is a Major Component of T-cell Dysfunction and Impedes Antitumor Immunity. Cancer Immunol Res 6: 14-24

Hosios AM, Vander Heiden MG (2014) Acetate metabolism in cancer cells. Cancer Metab 2: 27

Hou J, Greten TF, Xia Q (2017) Immunosuppressive cell death in cancer. Nat Rev Immunol 17: 401

Hou J, Zhou Y, Zheng Y, Fan J, Zhou W, Ng IO, Sun H, Qin L, Qiu S, Lee JM, Lo CM, Man K, Yang Y, Yang Y, Yang Y, Zhang Q, Zhu X, Li N, Wang Z, Ding G et al. (2014) Hepatic RIG-I predicts survival and interferon-alpha therapeutic response in hepatocellular carcinoma. Cancer Cell 25: 49-63

Howell JJ, Ricoult SJ, Ben-Sahra I, Manning BD (2013) A growing role for mTOR in promoting anabolic metabolism. Biochem Soc Trans 41: 906-12

Hoxhaj G, Hughes-Hallett J, Timson RC, Ilagan E, Yuan M, Asara JM, Ben-Sahra I, Manning BD (2017) The mTORC1 Signaling Network Senses Changes in Cellular Purine Nucleotide Levels. Cell Rep 21: 1331-1346

Hu F, Yu X, Wang H, Zuo D, Guo C, Yi H, Tirosh B, Subjeck JR, Qiu X, Wang XY (2011) ER stress and its regulator X-box-binding protein-1 enhance polyIC-induced innate immune response in dendritic cells. Eur J Immunol 41: 1086-97

Hu R, Warri A, Jin L, Zwart A, Riggins RB, Fang HB, Clarke R (2015) NF-kappaB signaling is required for XBP1 (unspliced and spliced)-mediated effects on antiestrogen responsiveness and cell fate decisions in breast cancer. Mol Cell Biol 35: 379-90

Huang J, Manning BD (2009) A complex interplay between Akt, TSC2 and the two mTOR complexes. Biochem Soc Trans 37: 217-22

Hur KY, So JS, Ruda V, Frank-Kamenetsky M, Fitzgerald K, Koteliansky V, Iwawaki T, Glimcher LH, Lee AH (2012) IRE1alpha activation protects mice against acetaminophen-induced hepatotoxicity. J Exp Med 209: 307-18

Imamura F, Micha R, Khatibzadeh S, Fahimi S, Shi P, Powles J, Mozaffarian D, Global Burden of Diseases N, Chronic Diseases Expert G (2015) Dietary quality among men and women in 187 countries in 1990 and 2010: a systematic assessment. Lancet Glob Health 3: e132-42

Ingram DK, Roth GS (2011) Glycolytic inhibition as a strategy for developing calorie restriction mimetics. Exp Gerontol 46: 148-54

Ingram DK, Roth GS (2015) Calorie restriction mimetics: can you have your cake and eat it, too? Ageing Res Rev 20: 46-62

Jeon SM, Chandel NS, Hay N (2012) AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. Nature 485: 661-5

Jin L, Alesi GN, Kang S (2016) Glutaminolysis as a target for cancer therapy. Oncogene 35: 3619-25

Johnson SC, Rabinovitch PS, Kaeberlein M (2013) mTOR is a key modulator of ageing and age-related disease. Nature 493: 338-45

Jones RB, O'Connor R, Mueller S, Foley M, Szeto GL, Karel D, Lichterfeld M, Kovacs C, Ostrowski MA, Trocha A, Irvine DJ, Walker BD (2014) Histone deacetylase inhibitors impair the elimination of HIV-infected cells by cytotoxic T-lymphocytes. PLoS Pathog 10: e1004287

Jones RG, Bui T, White C, Madesh M, Krawczyk CM, Lindsten T, Hawkins BJ, Kubek S, Frauwirth KA, Wang YL, Conway SJ, Roderick HL, Bootman MD, Shen H, Foskett JK, Thompson CB (2007) The proapoptotic factors Bax and Bak regulate T Cell proliferation through control of endoplasmic reticulum Ca(2+) homeostasis. Immunity 27: 268-80

Joyce JA, Fearon DT (2015) T cell exclusion, immune privilege, and the tumor microenvironment. Science 348: 74-80

Juneja VR, McGuire KA, Manguso RT, LaFleur MW, Collins N, Haining WN, Freeman GJ, Sharpe AH (2017) PD-L1 on tumor cells is sufficient for immune evasion in immunogenic tumors and inhibits CD8 T cell cytotoxicity. J Exp Med 214: 895-904

Jung H, Hsiung B, Pestal K, Procyk E, Raulet DH (2012) RAE-1 ligands for the NKG2D receptor are regulated by E2F transcription factors, which control cell cycle entry. J Exp Med 209: 2409-22

Kalaany NY, Sabatini DM (2009) Tumours with PI3K activation are resistant to dietary restriction. Nature 458: 725-31

Kalyanaraman B (2017) Teaching the basics of cancer metabolism: Developing antitumor strategies by exploiting the differences between normal and cancer cell metabolism. Redox Biol 12: 833-842

Kamphorst JJ, Chung MK, Fan J, Rabinowitz JD (2014) Quantitative analysis of acetyl-CoA production in hypoxic cancer cells reveals substantial contribution from acetate. Cancer Metab 2: 23

Katlinskaya YV, Katlinski KV, Yu Q, Ortiz A, Beiting DP, Brice A, Davar D, Sanders C, Kirkwood JM, Rui H, Xu X, Koumenis C, Diehl JA, Fuchs SY (2016) Suppression of Type I Interferon Signaling Overcomes Oncogene-Induced Senescence and Mediates Melanoma Development and Progression. Cell Rep 15: 171-180

Katlinski KV, Gui J, Katlinskaya YV, Ortiz A, Chakraborty R, Bhattacharya S, Carbone CJ, Beiting DP, Girondo MA, Peck AR, Pure E, Chatterji P, Rustgi AK, Diehl JA, Koumenis C, Rui H, Fuchs SY (2017) Inactivation of Interferon Receptor Promotes the Establishment of Immune Privileged Tumor Microenvironment. Cancer Cell 31: 194-207

Kawashima M, Ozawa Y, Shinmura K, Inaba T, Nakamura S, Kawakita T, Watanabe M, Tsubota K (2013) Calorie restriction (CR) and CR mimetics for the prevention and treatment of age-related eye disorders. Exp Gerontol 48: 1096-100

Kerndt PR, Naughton JL, Driscoll CE, Loxterkamp DA (1982) Fasting: the history, pathophysiology and complications. West J Med 137: 379-99

Kharazi AI, James SJ, Taylor JM, Lubinski JM, Nakamura LT, Makinodan T (1994) Combined chronic low dose radiation-caloric restriction: a model for regression of spontaneous mammary tumor. Int J Radiat Oncol Biol Phys 28: 641-7

Kim H, Bhattacharya A, Qi L (2015) Endoplasmic reticulum quality control in cancer: Friend or foe. Semin Cancer Biol 33: 25-33

Kim I, Xu W, Reed JC (2008) Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov 7: 1013-30

Kim J, Yang G, Kim Y, Kim J, Ha J (2016) AMPK activators: mechanisms of action and physiological activities. Exp Mol Med 48: e224

Kim JY, Garcia-Carbonell R, Yamachika S, Zhao P, Dhar D, Loomba R, Kaufman RJ, Saltiel AR, Karin M (2018) ER Stress Drives Lipogenesis and Steatohepatitis via Caspase-2 Activation of S1P. Cell 175: 133-145 e15

Kim M, Nitschke M, Sennino B, Murer P, Schriver BJ, Bell A, Subramanian A, McDonald CE, Wang J, Cha H, Bourgeois-Daigneault MC, Kirn DH, Bell JC, De Silva N, Breitbach CJ, McDonald DM (2018) Amplification of Oncolytic Vaccinia Virus Widespread Tumor Cell Killing by Sunitinib through Multiple Mechanisms. Cancer Res 78: 922-937

Kimura YN, Watari K, Fotovati A, Hosoi F, Yasumoto K, Izumi H, Kohno K, Umezawa K, Iguchi H, Shirouzu K, Takamori S, Kuwano M, Ono M (2007) Inflammatory stimuli from macrophages and cancer cells synergistically promote tumor growth and angiogenesis. Cancer Sci 98: 2009-18

Kitai Y, Ariyama H, Kono N, Oikawa D, Iwawaki T, Arai H (2013) Membrane lipid saturation activates IRE1alpha without inducing clustering. Genes Cells 18: 798-809

Kitajima S, Takahashi C (2017) Intersection of retinoblastoma tumor suppressor function, stem cells, metabolism, and inflammation. Cancer Sci 108: 1726-1731

Kitajima S, Yoshida A, Kohno S, Li F, Suzuki S, Nagatani N, Nishimoto Y, Sasaki N, Muranaka H, Wan Y, Thai TC, Okahashi N, Matsuda F, Shimizu H, Nishiuchi T, Suzuki Y, Tominaga K, Gotoh N, Suzuki M, Ewen ME et al. (2017) The RB-IL-6 axis controls self-renewal and endocrine therapy resistance by fine-tuning mitochondrial activity. Oncogene 36: 5145-5157

Klinke DJ, 2nd (2012) An evolutionary perspective on anti-tumor immunity. Front Oncol 2: 202

Krebs-Smith SM, Guenther PM, Subar AF, Kirkpatrick SI, Dodd KW (2010) Americans do not meet federal dietary recommendations. J Nutr 140: 1832-8

Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P (2012) Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer 12: 860-75

Kumar SP, Roy SJ, Tokumo K, Reddy BS (1990) Effect of different levels of calorie restriction on azoxymethane-induced colon carcinogenesis in male F344 rats. Cancer Res 50: 5761-6

Labunskyy VM, Gerashchenko MV, Delaney JR, Kaya A, Kennedy BK, Kaeberlein M, Gladyshev VN (2014) Lifespan extension conferred by endoplasmic reticulum secretory pathway deficiency requires induction of the unfolded protein response. PLoS Genet 10: e1004019

Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M, Stevens DM, Davis JG, Salmon AB, Richardson A, Ahima RS, Guertin DA, Sabatini DM, Baur JA (2012) Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science 335: 1638-43

Lancaster GI, Langley KG, Berglund NA, Kammoun HL, Reibe S, Estevez E, Weir J, Mellett NA, Pernes G, Conway JRW, Lee MKS, Timpson P, Murphy AJ, Masters SL, Gerondakis S, Bartonicek N, Kaczorowski DC, Dinger ME, Meikle PJ, Bond PJ et al. (2018) Evidence that TLR4 Is Not a Receptor for Saturated Fatty Acids but Mediates Lipid-Induced Inflammation by Reprogramming Macrophage Metabolism. Cell Metab 27: 1096-1110 e5

Landman GW, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, Bilo HJ (2010) Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. Diabetes Care 33: 322-6

Lane N, Martin W (2010) The energetics of genome complexity. Nature 467: 929-34

Lashinger LM, Malone LM, McArthur MJ, Goldberg JA, Daniels EA, Pavone A, Colby JK, Smith NC, Perkins SN, Fischer SM, Hursting SD (2011) Genetic reduction of insulin-like growth factor-1 mimics the anticancer effects of calorie restriction on cyclooxygenase-2-driven pancreatic neoplasia. Cancer Prev Res (Phila) 4: 1030-40

Lee C, Raffaghello L, Brandhorst S, Safdie FM, Bianchi G, Martin-Montalvo A, Pistoia V, Wei M, Hwang S, Merlino A, Emionite L, de Cabo R, Longo VD (2012) Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy. Sci Transl Med 4: 124ra27

Lee C, Safdie FM, Raffaghello L, Wei M, Madia F, Parrella E, Hwang D, Cohen P, Bianchi G, Longo VD (2010) Reduced levels of IGF-I mediate differential protection of normal and cancer cells in response to fasting and improve chemotherapeutic index. Cancer Res 70: 1564-72

Lee EB, Ahn D, Kim BJ, Lee SY, Seo HW, Cha YS, Jeon H, Eun JS, Cha DS, Kim DK (2015) Genistein from Vigna angularis Extends Lifespan in Caenorhabditis elegans. Biomol Ther (Seoul) 23: 77-83

Lee KP, Simpson SJ, Clissold FJ, Brooks R, Ballard JW, Taylor PW, Soran N, Raubenheimer D (2008) Lifespan and reproduction in Drosophila: New insights from nutritional geometry. Proc Natl Acad Sci U S A 105: 2498-503

Lee S, Margolin K (2011) Cytokines in cancer immunotherapy. Cancers (Basel) 3: 3856-93

Leprivier G, Remke M, Rotblat B, Dubuc A, Mateo AR, Kool M, Agnihotri S, El-Naggar A, Yu B, Somasekharan SP, Faubert B, Bridon G, Tognon CE, Mathers J, Thomas R, Li A, Barokas A, Kwok B, Bowden M, Smith S et al. (2013) The eEF2 kinase confers resistance to nutrient deprivation by blocking translation elongation. Cell 153: 1064-79

Levine ME, Suarez JA, Brandhorst S, Balasubramanian P, Cheng CW, Madia F, Fontana L, Mirisola MG, Guevara-Aguirre J, Wan J, Passarino G, Kennedy BK, Wei M, Cohen P, Crimmins EM, Longo VD (2014) Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. Cell Metab 19: 407-17

Li H, Chen X, Gao Y, Wu J, Zeng F, Song F (2015) XBP1 induces snail expression to promote epithelial- to-mesenchymal transition and invasion of breast cancer cells. Cell Signal 27: 82-9

Li HW, Zhao W, Sarkar NH (1994) Dietary regulation of mammary tumorigenesis in RIII/Sa mice: investigation of a possible mechanism. Cancer Lett 79: 199-211

Li J, Zhang CX, Liu YM, Chen KL, Chen G (2017a) A comparative study of anti-aging properties and mechanism: resveratrol and caloric restriction. Oncotarget 8: 65717-65729

Li K, Qu S, Chen X, Wu Q, Shi M (2017b) Promising Targets for Cancer Immunotherapy: TLRs, RLRs, and STING-Mediated Innate Immune Pathways. Int J Mol Sci 18

Li XX, Zhang HS, Xu YM, Zhang RJ, Chen Y, Fan L, Qin YQ, Liu Y, Li M, Fang J (2017c) Knockdown of IRE1alpha inhibits colonic tumorigenesis through decreasing beta-catenin and IRE1alpha targeting suppresses colon cancer cells. Oncogene

Liberti MV, Locasale JW (2016) The Warburg Effect: How Does it Benefit Cancer Cells? Trends Biochem Sci 41: 211-218

Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K (2000) Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 343: 78-85

Lim S, Phillips JB, Madeira da Silva L, Zhou M, Fodstad O, Owen LB, Tan M (2017) Interplay between Immune Checkpoint Proteins and Cellular Metabolism. Cancer Res 77: 1245-1249

Lippens C, Duraes FV, Dubrot J, Brighouse D, Lacroix M, Irla M, Aubry-Lachainaye JP, Reith W, Mandl JN, Hugues S (2016) IDO-orchestrated crosstalk between pDCs and Tregs inhibits autoimmunity. J Autoimmun 75: 39-49

Liu Y, Komohara Y, Domenick N, Ohno M, Ikeura M, Hamilton RL, Horbinski C, Wang X, Ferrone S, Okada H (2012) Expression of antigen processing and presenting molecules in brain metastasis of breast cancer. Cancer Immunol Immunother 61: 789-801

Llambi F, Wang YM, Victor B, Yang M, Schneider DM, Gingras S, Parsons MJ, Zheng JH, Brown SA, Pelletier S, Moldoveanu T, Chen T, Green DR (2016) BOK Is a Non-canonical BCL-2 Family Effector of Apoptosis Regulated by ER-Associated Degradation. Cell 165: 421-33

Lo Presti E, Pizzolato G, Corsale AM, Caccamo N, Sireci G, Dieli F, Meraviglia S (2018) gammadelta T Cells and Tumor Microenvironment: From Immunosurveillance to Tumor Evasion. Front Immunol 9: 1395

Logue SE, McGrath EP, Cleary P, Greene S, Mnich K, Almanza A, Chevet E, Dwyer RM, Oommen A, Legembre P, Godey F, Madden EC, Leuzzi B, Obacz J, Zeng Q, Patterson JB, Jager R, Gorman AM, Samali A (2018) Inhibition of IRE1 RNase activity modulates the tumor cell secretome and enhances response to chemotherapy. Nat Commun 9: 3267

Ma EH, Bantug G, Griss T, Condotta S, Johnson RM, Samborska B, Mainolfi N, Suri V, Guak H, Balmer ML, Verway MJ, Raissi TC, Tsui H, Boukhaled G, Henriques da Costa S, Frezza C, Krawczyk CM, Friedman A, Manfredi M, Richer MJ et al. (2017) Serine Is an Essential Metabolite for Effector T Cell Expansion. Cell Metab 25: 345-357

Ma Y, Hendershot LM (2004) The role of the unfolded protein response in tumour development: friend or foe? Nat Rev Cancer 4: 966-77

Maddocks ODK, Athineos D, Cheung EC, Lee P, Zhang T, van den Broek NJF, Mackay GM, Labuschagne CF, Gay D, Kruiswijk F, Blagih J, Vincent DF, Campbell KJ, Ceteci F, Sansom OJ, Blyth K, Vousden KH (2017) Modulating the therapeutic response of tumours to dietary serine and glycine starvation. Nature 544: 372-376

Madeo F, Pietrocola F, Eisenberg T, Kroemer G (2014) Caloric restriction mimetics: towards a molecular definition. Nat Rev Drug Discov 13: 727-40

Mahnke YD, Devevre E, Baumgaertner P, Matter M, Rufer N, Romero P, Speiser DE (2012) Human melanoma-specific CD8(+) T-cells from metastases are capable of antigen-specific degranulation and cytolysis directly ex vivo. Oncoimmunology 1: 467-530

Mai V, Colbert LH, Berrigan D, Perkins SN, Pfeiffer R, Lavigne JA, Lanza E, Haines DC, Schatzkin A, Hursting SD (2003) Calorie restriction and diet composition modulate spontaneous intestinal tumorigenesis in Apc(Min) mice through different mechanisms. Cancer Res 63: 1752-5

Malamas AS, Gameiro SR, Knudson KM, Hodge JW (2016) Sublethal exposure to alpha radiation (223Ra dichloride) enhances various carcinomas' sensitivity to lysis by antigen-specific cytotoxic T lymphocytes through calreticulin-mediated immunogenic modulation. Oncotarget 7: 86937-86947

Manning BD (2017) Game of TOR - The Target of Rapamycin Rules Four Kingdoms. N Engl J Med 377: 1297-9

Mantovani A, Sica A (2010) Macrophages, innate immunity and cancer: balance, tolerance, and diversity. Curr Opin Immunol 22: 231-7

Marcu MG, Jung YJ, Lee S, Chung EJ, Lee MJ, Trepel J, Neckers L (2006) Curcumin is an inhibitor of p300 histone acetylatransferase. Med Chem 2: 169-74

Marcus A, Gowen BG, Thompson TW, Iannello A, Ardolino M, Deng W, Wang L, Shifrin N, Raulet DH (2014) Recognition of tumors by the innate immune system and natural killer cells. Adv Immunol 122: 91-128

Marino G, Pietrocola F, Madeo F, Kroemer G (2014) Caloric restriction mimetics: natural/physiological pharmacological autophagy inducers. Autophagy 10: 1879-82

Mark KA, Dumas KJ, Bhaumik D, Schilling B, Davis S, Oron TR, Sorensen DJ, Lucanic M, Brem RB, Melov S, Ramanathan A, Gibson BW, Lithgow GJ (2016) Vitamin D Promotes Protein Homeostasis and Longevity via the Stress Response Pathway Genes skn-1, ire-1, and xbp-1. Cell Rep 17: 1227-1237

Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, Gomes AP, Ward TM, Minor RK, Blouin MJ, Schwab M, Pollak M, Zhang Y, Yu Y, Becker KG, Bohr VA, Ingram DK, Sinclair DA, Wolf NS, Spindler SR et al. (2013) Metformin improves healthspan and lifespan in mice. Nat Commun 4: 2192

Martinon F, Chen X, Lee AH, Glimcher LH (2010) TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. Nat Immunol 11: 411-8

Martins I, Kepp O, Schlemmer F, Adjemian S, Tailler M, Shen S, Michaud M, Menger L, Gdoura A, Tajeddine N, Tesniere A, Zitvogel L, Kroemer G (2011) Restoration of the immunogenicity of cisplatin-induced cancer cell death by endoplasmic reticulum stress. Oncogene 30: 1147-58

Mattarollo SR, Loi S, Duret H, Ma Y, Zitvogel L, Smyth MJ (2011) Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. Cancer Res 71: 4809-20

Maurel M, Chevet E, Tavernier J, Gerlo S (2014) Getting RIDD of RNA: IRE1 in cell fate regulation. Trends Biochem Sci 39: 245-54

McCarty MF, Block KI (2006) Preadministration of high-dose salicylates, suppressors of NF-kappaB activation, may increase the chemosensitivity of many cancers: an example of proapoptotic signal modulation therapy. Integr Cancer Ther 5: 252-68

McCay CM, Crowell MF, Maynard LA (1989) The effect of retarded growth upon the length of life span and upon the ultimate body size. 1935. Nutrition 5: 155-71; discussion 172

McNeil JJ, Nelson MR, Woods RL, Lockery JE, Wolfe R, Reid CM, Kirpach B, Shah RC, Ives DG, Storey E, Ryan J, Tonkin AM, Newman AB, Williamson JD, Margolis KL, Ernst ME, Abhayaratna WP, Stocks N, Fitzgerald SM, Orchard SG et al. (2018) Effect of Aspirin on All-Cause Mortality in the Healthy Elderly. N Engl J Med

Menger L, Vacchelli E, Adjemian S, Martins I, Ma Y, Shen S, Yamazaki T, Sukkurwala AQ, Michaud M, Mignot G, Schlemmer F, Sulpice E, Locher C, Gidrol X, Ghiringhelli F, Modjtahedi N, Galluzzi L, Andre F, Zitvogel L, Kepp O et al. (2012) Cardiac glycosides exert anticancer effects by inducing immunogenic cell death. Sci Transl Med 4: 143ra99

Meynet O, Beneteau M, Jacquin MA, Pradelli LA, Cornille A, Carles M, Ricci JE (2012) Glycolysis inhibition targets Mcl-1 to restore sensitivity of lymphoma cells to ABT-737-induced apoptosis. Leukemia 26: 1145-7

Meynet O, Ricci JE (2014) Caloric restriction and cancer: molecular mechanisms and clinical implications. Trends Mol Med 20: 419-27

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, et al. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66-71

Minor RK, Smith DL, Jr., Sossong AM, Kaushik S, Poosala S, Spangler EL, Roth GS, Lane M, Allison DB, de Cabo R, Ingram DK, Mattison JA (2010) Chronic ingestion of 2-deoxy-D-glucose induces cardiac vacuolization and increases mortality in rats. Toxicol Appl Pharmacol 243: 332-9

Mitchell SJ, Bernier M, Mattison JA, Aon MA, Kaiser TA, Anson RM, Ikeno Y, Anderson RM, Ingram DK, de Cabo R (2018) Daily Fasting Improves Health and Survival in Male Mice Independent of Diet Composition and Calories. Cell Metab

Mittal D, Gubin MM, Schreiber RD, Smyth MJ (2014) New insights into cancer immunoediting and its three component phases--elimination, equilibrium and escape. Curr Opin Immunol 27: 16-25

Monge J, Kricun M, Radovcic J, Radovcic D, Mann A, Frayer DW (2013) Fibrous dysplasia in a 120,000+ year old Neandertal from Krapina, Croatia. PLoS One 8: e64539

Monu NR, Frey AB (2012) Myeloid-derived suppressor cells and anti-tumor T cells: a complex relationship. Immunol Invest 41: 595-613

Moore LV, Dodd KW, Thompson FE, Grimm KA, Kim SA, Scanlon KS (2015) Using Behavioral Risk Factor Surveillance System Data to Estimate the Percentage of the Population Meeting US Department of Agriculture Food Patterns Fruit and Vegetable Intake Recommendations. Am J Epidemiol 181: 979-88

Moyer JD, Oliver JT, Handschumacher RE (1981) Salvage of circulating pyrimidine nucleosides in the rat. Cancer Res 41: 3010-7

Mujcic H, Nagelkerke A, Rouschop KM, Chung S, Chaudary N, Span PN, Clarke B, Milosevic M, Sykes J, Hill RP, Koritzinsky M, Wouters BG (2013) Hypoxic activation of the PERK/eIF2alpha arm of the unfolded protein response promotes metastasis through induction of LAMP3. Clin Cancer Res 19: 6126-37

Munoz JP, Ivanova S, Sanchez-Wandelmer J, Martinez-Cristobal P, Noguera E, Sancho A, Diaz-Ramos A, Hernandez-Alvarez MI, Sebastian D, Mauvezin C, Palacin M, Zorzano A (2013) Mfn2 modulates the UPR and mitochondrial function via repression of PERK. EMBO J 32: 2348-61

Nabet BY, Qiu Y, Shabason JE, Wu TJ, Yoon T, Kim BC, Benci JL, DeMichele AM, Tchou J, Marcotrigiano J, Minn AJ (2017) Exosome RNA Unshielding Couples Stromal Activation to Pattern Recognition Receptor Signaling in Cancer. Cell 170: 352-366 e13

Naidoo N, Ferber M, Master M, Zhu Y, Pack AI (2008) Aging impairs the unfolded protein response to sleep deprivation and leads to proapoptotic signaling. J Neurosci 28: 6539-48

Naito Y, Saito K, Shiiba K, Ohuchi A, Saigenji K, Nagura H, Ohtani H (1998) CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res 58: 3491-4

Nakagawa H, Umemura A, Taniguchi K, Font-Burgada J, Dhar D, Ogata H, Zhong Z, Valasek MA, Seki E, Hidalgo J, Koike K, Kaufman RJ, Karin M (2014) ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. Cancer Cell 26: 331-343

Nakamura A, Nambu T, Ebara S, Hasegawa Y, Toyoshima K, Tsuchiya Y, Tomita D, Fujimoto J, Kurasawa O, Takahara C, Ando A, Nishigaki R, Satomi Y, Hata A, Hara T (2018) Inhibition of GCN2 sensitizes ASNS-low cancer cells to asparaginase by disrupting the amino acid response. Proc Natl Acad Sci U S A 115: E7776-E7785

Namba T, Chu K, Kodama R, Byun S, Yoon KW, Hiraki M, Mandinova A, Lee SW (2015) Loss of p53 enhances the function of the endoplasmic reticulum through activation of the IRE1alpha/XBP1 pathway. Oncotarget 6: 19990-20001

Niederreiter L, Fritz TM, Adolph TE, Krismer AM, Offner FA, Tschurtschenthaler M, Flak MB, Hosomi S, Tomczak MF, Kaneider NC, Sarcevic E, Kempster SL, Raine T, Esser D, Rosenstiel P, Kohno K, Iwawaki T, Tilg H, Blumberg RS, Kaser A (2013) ER stress transcription factor Xbp1 suppresses intestinal tumorigenesis and directs intestinal stem cells. J Exp Med 210: 2041-56

Nikolai S, Pallauf K, Huebbe P, Rimbach G (2015) Energy restriction and potential energy restriction mimetics. Nutr Res Rev 28: 100-120

Niu Z, Wang M, Zhou L, Yao L, Liao Q, Zhao Y (2015) Elevated GRP78 expression is associated with poor prognosis in patients with pancreatic cancer. Sci Rep 5: 16067

Norman C, Howell KA, Millar AH, Whelan JM, Day DA (2004) Salicylic acid is an uncoupler and inhibitor of mitochondrial electron transport. Plant Physiol 134: 492-501

Nowarski R, Gagliani N, Huber S, Flavell RA (2013) Innate immune cells in inflammation and cancer. Cancer Immunol Res 1: 77-84

Noy R, Pollard JW (2014) Tumor-associated macrophages: from mechanisms to therapy. Immunity 41: 49-61

O'Sullivan T, Saddawi-Konefka R, Vermi W, Koebel CM, Arthur C, White JM, Uppaluri R, Andrews DM, Ngiow SF, Teng MW, Smyth MJ, Schreiber RD, Bui JD (2012) Cancer immunoediting by the innate immune system in the absence of adaptive immunity. J Exp Med 209: 1869-82

Obacz J, Avril T, Le Reste PJ, Urra H, Quillien V, Hetz C, Chevet E (2017) Endoplasmic reticulum proteostasis in glioblastoma-From molecular mechanisms to therapeutic perspectives. Sci Signal 10

Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, Castedo M, Mignot G, Panaretakis T, Casares N, Metivier D, Larochette N, van Endert P, Ciccosanti F, Piacentini M, Zitvogel L, Kroemer G (2007) Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med 13: 54-61

Orillion AR, Damayanti NP, Shen L, Adelaiye-Ogala R, Affronti HC, Elbanna M, Chintala S, Ciesielski MJ, Fontana L, Kao C, Elzey BD, Ratliff TL, Nelson DE, Smiraglia DJ, Abrams SI, Pili R (2018) Dietary protein restriction reprograms tumor associated macrophages and enhances immunotherapy. Clin Cancer Res

Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J (2005) Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 353: 2654-66

Pallauf K, Rimbach G (2013) Autophagy, polyphenols and healthy ageing. Ageing Res Rev 12: 237-52

Pallavi R, Giorgio M, Pelicci PG (2012) Insights into the beneficial effect of caloric/ dietary restriction for a healthy and prolonged life. Front Physiol 3: 318

Panaretakis T, Kepp O, Brockmeier U, Tesniere A, Bjorklund AC, Chapman DC, Durchschlag M, Joza N, Pierron G, van Endert P, Yuan J, Zitvogel L, Madeo F, Williams DB, Kroemer G (2009) Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. EMBO J 28: 578-90

Papaioannou NE, Beniata OV, Vitsos P, Tsitsilonis O, Samara P (2016) Harnessing the immune system to improve cancer therapy. Ann Transl Med 4: 261

Park IA, Heo SH, Song IH, Kim YA, Park HS, Bang WS, Park SY, Jo JH, Lee HJ, Gong G (2016) Endoplasmic reticulum stress induces secretion of high-mobility group proteins and is associated with tumor-infiltrating lymphocytes in triple-negative breast cancer. Oncotarget 7: 59957-59964

Paul-Clark MJ, Van Cao T, Moradi-Bidhendi N, Cooper D, Gilroy DW (2004) 15-epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. J Exp Med 200: 69-78

Pavlova NN, Thompson CB (2016) The Emerging Hallmarks of Cancer Metabolism. Cell Metab 23: 27-47

Pereira ER, Frudd K, Awad W, Hendershot LM (2014) Endoplasmic reticulum (ER) stress and hypoxia response pathways interact to potentiate hypoxia-inducible factor 1 (HIF-1) transcriptional activity on targets like vascular endothelial growth factor (VEGF). J Biol Chem 289: 3352-64

Perez-Escuredo J, Dadhich RK, Dhup S, Cacace A, Van Hee VF, De Saedeleer CJ, Sboarina M, Rodriguez F, Fontenille MJ, Brisson L, Porporato PE, Sonveaux P (2016) Lactate promotes glutamine uptake and metabolism in oxidative cancer cells. Cell Cycle 15: 72-83

Pernicova I, Korbonits M (2014) Metformin--mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol 10: 143-56

Peterson LW, Artis D (2014) Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 14: 141-53

Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, Yamazaki T, Poirier-Colame V, Newton A, Redouane Y, Lin YJ, Wojtkiewicz G, Iwamoto Y, Mino-Kenudson M, Huynh TG, Hynes RO, Freeman GJ, Kroemer G, Zitvogel L, Weissleder R et al. (2016) Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy. Immunity 44: 343-54

Phoenix KN, Vumbaca F, Fox MM, Evans R, Claffey KP (2010) Dietary energy availability affects primary and metastatic breast cancer and metformin efficacy. Breast Cancer Res Treat 123: 333-44

Pietrocola F, Castoldi F, Maiuri MC, Kroemer G (2018a) Aspirin-another caloric-restriction mimetic. Autophagy: 1-2

Pietrocola F, Castoldi F, Markaki M, Lachkar S, Chen G, Enot DP, Durand S, Bossut N, Tong M, Malik SA, Loos F, Dupont N, Marino G, Abdelkader N, Madeo F, Maiuri MC, Kroemer R, Codogno P, Sadoshima J, Tavernarakis N et al. (2018b) Aspirin Recapitulates Features of Caloric Restriction. Cell Rep 22: 2395-2407

Pietrocola F, Pol J, Vacchelli E, Rao S, Enot DP, Baracco EE, Levesque S, Castoldi F, Jacquelot N, Yamazaki T, Senovilla L, Marino G, Aranda F, Durand S, Sica V, Chery A, Lachkar S, Sigl V, Bloy N, Buque A et al. (2016) Caloric Restriction Mimetics Enhance Anticancer Immunosurveillance. Cancer Cell 30: 147-160

Piperi C, Samaras V, Levidou G, Kavantzas N, Boviatsis E, Petraki K, Grivas A, Barbatis C, Varsos V, Patsouris E, Korkolopoulou P (2011) Prognostic significance of IL-8-STAT-3 pathway in astrocytomas: correlation with IL-6, VEGF and microvessel morphometry. Cytokine 55: 387-95

Ploeger JM, Manivel JC, Boatner LN, Mashek DG (2017) Caloric Restriction Prevents Carcinogen-Initiated Liver Tumorigenesis in Mice. Cancer Prev Res (Phila) 10: 660-670

Poeck H, Besch R, Maihoefer C, Renn M, Tormo D, Morskaya SS, Kirschnek S, Gaffal E, Landsberg J, Hellmuth J, Schmidt A, Anz D, Bscheider M, Schwerd T, Berking C, Bourquin C, Kalinke U, Kremmer E, Kato H, Akira S et al. (2008) 5'-Triphosphate-siRNA: turning gene silencing and Rig-I activation against melanoma. Nat Med 14: 1256-63

Puthalakath H, O'Reilly LA, Gunn P, Lee L, Kelly PN, Huntington ND, Hughes PD, Michalak EM, McKimm-Breschkin J, Motoyama N, Gotoh T, Akira S, Bouillet P, Strasser A (2007) ER stress triggers apoptosis by activating BH3-only protein Bim. Cell 129: 1337-49

Pytel D, Majsterek I, Diehl JA (2016) Tumor progression and the different faces of the PERK kinase. Oncogene 35: 1207-15

Raghavan M, Wijeyesakere SJ, Peters LR, Del Cid N (2013) Calreticulin in the immune system: ins and outs. Trends Immunol 34: 13-21

Ramadori G, Konstantinidou G, Venkateswaran N, Biscotti T, Morlock L, Galie M, Williams NS, Luchetti M, Santinelli A, Scaglioni PP, Coppari R (2015) Diet-induced unresolved ER stress hinders KRAS-driven lung tumorigenesis. Cell Metab 21: 117-25

Ramakrishnan R, Tyurin VA, Veglia F, Condamine T, Amoscato A, Mohammadyani D, Johnson JJ, Zhang LM, Klein-Seetharaman J, Celis E, Kagan VE, Gabrilovich DI (2014) Oxidized lipids block antigen cross-presentation by dendritic cells in cancer. J Immunol 192: 2920-31

Ranganathan AC, Zhang L, Adam AP, Aguirre-Ghiso JA (2006) Functional coupling of p38-induced up-regulation of BiP and activation of RNA-dependent protein kinase-like endoplasmic reticulum kinase to drug resistance of dormant carcinoma cells. Cancer Res 66: 1702-11

Rena G, Hardie DG, Pearson ER (2017) The mechanisms of action of metformin. Diabetologia 60: 1577-1585

Reyes R, Wani NA, Ghoshal K, Jacob ST, Motiwala T (2017) Sorafenib and 2-Deoxyglucose Synergistically Inhibit Proliferation of Both Sorafenib-Sensitive and -Resistant HCC Cells by Inhibiting ATP Production. Gene Expr 17: 129-140

Richter M, Vidovic N, Honrath B, Mahavadi P, Dodel R, Dolga AM, Culmsee C (2016) Activation of SK2 channels preserves ER Ca(2)(+) homeostasis and protects against ER stress-induced cell death. Cell Death Differ 23: 814-27

Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, Runza V, Rey-Giraud F, Pradel LP, Feuerhake F, Klaman I, Jones T, Jucknischke U, Scheiblich S, Kaluza K, Gorr IH, Walz A, Abiraj K, Cassier PA, Sica A, Gomez-Roca C et al. (2014) Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. Cancer Cell 25: 846-59

Robien K, Demark-Wahnefried W, Rock CL (2011) Evidence-based nutrition guidelines for cancer survivors: current guidelines, knowledge gaps, and future research directions. J Am Diet Assoc 111: 368-75

Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, Delgado A, Correa P, Brayer J, Sotomayor EM, Antonia S, Ochoa JB, Ochoa AC (2004) Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. Cancer Res 64: 5839-49

Roebuck BD, Baumgartner KJ, MacMillan DL (1993) Caloric restriction and intervention in pancreatic carcinogenesis in the rat. Cancer Res 53: 46-52

Rothschild BM, Witzke BJ, Hershkovitz I (1999) Metastatic cancer in the Jurassic. Lancet 354: 398

Rubio-Patino C, Bossowski JP, Chevet E, Ricci JE (2018a) Reshaping the Immune Tumor Microenvironment Through IRE1 Signaling. Trends Mol Med 24: 607-614

Rubio-Patino C, Bossowski JP, De Donatis GM, Mondragon L, Villa E, Aira LE, Chiche J, Mhaidly R, Lebeaupin C, Marchetti S, Voutetakis K, Chatziioannou A, Castelli FA, Lamourette P, Chu-Van E, Fenaille F, Avril T, Passeron T, Patterson JB, Verhoeyen E et al. (2018b) Low-Protein Diet Induces IRE1alpha-Dependent Anticancer Immunosurveillance. Cell Metab 27: 828-842 e7

Rubio-Patino C, Bossowski JP, Villa E, Mondragon L, Zunino B, Proics E, Chiche J, Bost F, Verhoeyen E, Ricci JE (2016) Low carbohydrate diet prevents Mcl-1-mediated resistance to BH3-mimetics. Oncotarget 7: 73270-73279

Ruegsegger GN, Sevage JA, Childs TE, Grigsby KB, Booth FW (2017) 5-Aminoimidazole-4-carboxamide ribonucleotide prevents fat gain following the cessation of voluntary physical activity. Exp Physiol 102: 1474-1485

Ruggeri BA, Klurfeld DM, Kritchevsky D, Furlanetto RW (1989) Caloric restriction and 7,12-dimethylbenz(a)anthracene-induced mammary tumor growth in rats: alterations in circulating insulin, insulin-like growth factors I and II, and epidermal growth factor. Cancer Res 49: 4130-4

Sabatini DM (2017) Twenty-five years of mTOR: Uncovering the link from nutrients to growth. Proc Natl Acad Sci U S A 114: 11818-11825

Sahu N, Dela Cruz D, Gao M, Sandoval W, Haverty PM, Liu J, Stephan JP, Haley B, Classon M, Hatzivassiliou G, Settleman J (2016) Proline Starvation Induces Unresolved ER Stress and Hinders mTORC1-Dependent Tumorigenesis. Cell Metab 24: 753-761

Saintigny P, Massarelli E, Lin S, Ahn YH, Chen Y, Goswami S, Erez B, O'Reilly MS, Liu D, Lee JJ, Zhang L, Ping Y, Behrens C, Solis Soto LM, Heymach JV, Kim ES, Herbst RS, Lippman SM, Wistuba, II, Hong WK et al. (2013) CXCR2 expression in tumor cells is a poor prognostic factor and promotes invasion and metastasis in lung adenocarcinoma. Cancer Res 73: 571-82

Sakatani T, Maemura K, Hiyama N, Amano Y, Watanabe K, Kage H, Fukayama M, Nakajima J, Yatomi Y, Nagase T, Takai D (2017) High expression of IRE1 in lung adenocarcinoma is associated with a lower rate of recurrence. Jpn J Clin Oncol 47: 543-550

Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM (2010) Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 141: 290-303

Sanmamed MF, Chen L (2014) Inducible expression of B7-H1 (PD-L1) and its selective role in tumor site immune modulation. Cancer J 20: 256-61

Sarker MR, Franks S, Sumien N, Thangthaeng N, Filipetto F, Forster M (2015) Curcumin Mimics the Neurocognitive and Anti-Inflammatory Effects of Caloric Restriction in a Mouse Model of Midlife Obesity. PLoS One 10: e0140431

Sayin VI, LeBoeuf SE, Singh SX, Davidson SM, Biancur D, Guzelhan BS, Alvarez SW, Wu WL, Karakousi TR, Zavitsanou AM, Ubriaco J, Muir A, Karagiannis D, Morris PJ, Thomas CJ, Possemato R, Vander Heiden MG, Papagiannakopoulos T (2017) Activation of the NRF2 antioxidant program generates an imbalance in central carbon metabolism in cancer. Elife 6

Schewe DM, Aguirre-Ghiso JA (2008) ATF6alpha-Rheb-mTOR signaling promotes survival of dormant tumor cells in vivo. Proc Natl Acad Sci U S A 105: 10519-24

Schmelzle T, Hall MN (2000) TOR, a central controller of cell growth. Cell 103: 253-62

Schonthal AH (2012) Endoplasmic reticulum stress: its role in disease and novel prospects for therapy. Scientifica (Cairo) 2012: 857516

Scortegagna M, Kim H, Li JL, Yao H, Brill LM, Han J, Lau E, Bowtell D, Haddad G, Kaufman RJ, Ronai ZA (2014) Fine tuning of the UPR by the ubiquitin ligases Siah1/2. PLoS Genet 10: e1004348

Seliger B, Maeurer MJ, Ferrone S (2000) Antigen-processing machinery breakdown and tumor growth. Immunol Today 21: 455-64

Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, Galluzzi L, Adjemian S, Kepp O, Niso-Santano M, Shen S, Marino G, Criollo A, Boileve A, Job B, Ladoire S, Ghiringhelli F, Sistigu A, Yamazaki T, Rello-Varona S et al. (2012) An immunosurveillance mechanism controls cancer cell ploidy. Science 337: 1678-84

Shalapour S, Karin M (2015) Immunity, inflammation, and cancer: an eternal fight between good and evil. J Clin Invest 125: 3347-55

Shanware NP, Bray K, Eng CH, Wang F, Follettie M, Myers J, Fantin VR, Abraham RT (2014) Glutamine deprivation stimulates mTOR-JNK-dependent chemokine secretion. Nat Commun 5: 4900

Shi Y, Felley-Bosco E, Marti TM, Orlowski K, Pruschy M, Stahel RA (2012) Starvation-induced activation of ATM/Chk2/p53 signaling sensitizes cancer cells to cisplatin. BMC Cancer 12: 571

Shin CS, Mishra P, Watrous JD, Carelli V, D'Aurelio M, Jain M, Chan DC (2017) The glutamate/cystine xCT antiporter antagonizes glutamine metabolism and reduces nutrient flexibility. Nat Commun 8: 15074

Simpson SJ, Le Couteur DG, James DE, George J, Gunton JE, Solon-Biet SM, Raubenheimer D (2017) The Geometric Framework for Nutrition as a tool in precision medicine. Nutr Healthy Aging 4: 217-226

Sinclair LV, Rolf J, Emslie E, Shi YB, Taylor PM, Cantrell DA (2013) Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. Nat Immunol 14: 500-8

Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, Vitale I, Goubar A, Baracco EE, Remedios C, Fend L, Hannani D, Aymeric L, Ma Y, Niso-Santano M, Kepp O, Schultze JL, Tuting T, Belardelli F, Bracci L et al. (2014) Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med 20: 1301-9

Smith DL, Jr., Elam CF, Jr., Mattison JA, Lane MA, Roth GS, Ingram DK, Allison DB (2010) Metformin supplementation and life span in Fischer-344 rats. J Gerontol A Biol Sci Med Sci 65: 468-74

Smith JA (2014) A new paradigm: innate immune sensing of viruses via the unfolded protein response. Front Microbiol 5: 222

Smith JA, Turner MJ, DeLay ML, Klenk EI, Sowders DP, Colbert RA (2008) Endoplasmic reticulum stress and the unfolded protein response are linked to synergistic IFN-beta induction via X-box binding protein 1. Eur J Immunol 38: 1194-203

Solon-Biet SM, McMahon AC, Ballard JW, Ruohonen K, Wu LE, Cogger VC, Warren A, Huang X, Pichaud N, Melvin RG, Gokarn R, Khalil M, Turner N, Cooney GJ, Sinclair DA, Raubenheimer D, Le Couteur DG, Simpson SJ

(2014) The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. Cell Metab 19: 418-30

Solon-Biet SM, Mitchell SJ, Coogan SC, Cogger VC, Gokarn R, McMahon AC, Raubenheimer D, de Cabo R, Simpson SJ, Le Couteur DG (2015) Dietary Protein to Carbohydrate Ratio and Caloric Restriction: Comparing Metabolic Outcomes in Mice. Cell Rep 11: 1529-34

Song M, Giovannucci EL (2015a) Cancer risk: many factors contribute. Science 347: 728-9

Song M, Giovannucci EL (2015b) RE: Doll and Peto's Quantitative Estimates of Cancer Risks: Holding Generally True for 35 Years. J Natl Cancer Inst 107

Speakman JR, Mitchell SE, Mazidi M (2016) Calories or protein? The effect of dietary restriction on lifespan in rodents is explained by calories alone. Exp Gerontol 86: 28-38

Spisek R, Charalambous A, Mazumder A, Vesole DH, Jagannath S, Dhodapkar MV (2007) Bortezomib enhances dendritic cell (DC)-mediated induction of immunity to human myeloma via exposure of cell surface heat shock protein 90 on dying tumor cells: therapeutic implications. Blood 109: 4839-45

Spranger S (2016) Mechanisms of tumor escape in the context of the T-cell-inflamed and the non-T-cell-inflamed tumor microenvironment. Int Immunol 28: 383-91

Sriburi R, Bommiasamy H, Buldak GL, Robbins GR, Frank M, Jackowski S, Brewer JW (2007) Coordinate regulation of phospholipid biosynthesis and secretory pathway gene expression in XBP-1(S)-induced endoplasmic reticulum biogenesis. J Biol Chem 282: 7024-34

Stemmermann GN, Nomura AM, Chyou PH, Kato I, Kuroishi T (1991) Cancer incidence in Hawaiian Japanese: migrants from Okinawa compared with those from other prefectures. Jpn J Cancer Res 82: 1366-70

Stowell SR, Ju T, Cummings RD (2015) Protein glycosylation in cancer. Annu Rev Pathol 10: 473-510

Sukkurwala AQ, Martins I, Wang Y, Schlemmer F, Ruckenstuhl C, Durchschlag M, Michaud M, Senovilla L, Sistigu A, Ma Y, Vacchelli E, Sulpice E, Gidrol X, Zitvogel L, Madeo F, Galluzzi L, Kepp O, Kroemer G (2014) Immunogenic calreticulin exposure occurs through a phylogenetically conserved stress pathway involving the chemokine CXCL8. Cell Death Differ 21: 59-68

Sullivan MR, Vander Heiden MG (2017) When cancer needs what's non-essential. Nat Cell Biol 19: 418-420

Swamy M, Pathak S, Grzes KM, Damerow S, Sinclair LV, van Aalten DM, Cantrell DA (2016) Glucose and glutamine fuel protein O-GlcNAcylation to control T cell self-renewal and malignancy. Nat Immunol 17: 712-20

Swindell WR (2009) Heat shock proteins in long-lived worms and mice with insulin/insulin-like signaling mutations. Aging (Albany NY) 1: 573-7

Talapatra S, Thompson CB (2001) Growth factor signaling in cell survival: implications for cancer treatment. J Pharmacol Exp Ther 298: 873-8

Tam AB, Koong AC, Niwa M (2014) Ire1 has distinct catalytic mechanisms for XBP1/HAC1 splicing and RIDD. Cell Rep 9: 850-8

Tang J, Guo YS, Zhang Y, Yu XL, Li L, Huang W, Li Y, Chen B, Jiang JL, Chen ZN (2012) CD147 induces UPR to inhibit apoptosis and chemosensitivity by increasing the transcription of Bip in hepatocellular carcinoma. Cell Death Differ 19: 1779-90

Tannenbaum A, Silverstone H (1953) Effect of limited food intake on survival of mice bearing spontaneous mammary carcinoma and on the incidence of lung metastases. Cancer Res 13: 532-6

Tauxe W (2015) A tumour through time. Nature 527: S102-3

Taylor RC, Dillin A (2013) XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. Cell 153: 1435-47

Terranova-Barberio M, Thomas S, Ali N, Pawlowska N, Park J, Krings G, Rosenblum MD, Budillon A, Munster PN (2017) HDAC inhibition potentiates immunotherapy in triple negative breast cancer. Oncotarget 8: 114156-114172

Thompson CB (2011) Rethinking the regulation of cellular metabolism. Cold Spring Harb Symp Quant Biol 76: 23-9

Tsuru A, Imai Y, Saito M, Kohno K (2016) Novel mechanism of enhancing IRE1alpha-XBP1 signalling via the PERK-ATF4 pathway. Sci Rep 6: 24217

Tyrakis PA, Palazon A, Macias D, Lee KL, Phan AT, Velica P, You J, Chia GS, Sim J, Doedens A, Abelanet A, Evans CE, Griffiths JR, Poellinger L, Goldrath AW, Johnson RS (2016) S-2-hydroxyglutarate regulates CD8(+) T-lymphocyte fate. Nature 540: 236-241

Ulianich L, Terrazzano G, Annunziatella M, Ruggiero G, Beguinot F, Di Jeso B (2011) ER stress impairs MHC Class I surface expression and increases susceptibility of thyroid cells to NK-mediated cytotoxicity. Biochim Biophys Acta 1812: 431-8

Urra H, Dufey E, Avril T, Chevet E, Hetz C (2016) Endoplasmic Reticulum Stress and the Hallmarks of Cancer. Trends Cancer 2: 252-262

Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, Boon T, Van den Eynde BJ (2003) Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med 9: 1269-74

Valastyan S, Weinberg RA (2011) Tumor metastasis: molecular insights and evolving paradigms. Cell 147: 275-92

Valvezan AJ, Turner M, Belaid A, Lam HC, Miller SK, McNamara MC, Baglini C, Housden BE, Perrimon N, Kwiatkowski DJ, Asara JM, Henske EP, Manning BD (2017) mTORC1 Couples Nucleotide Synthesis to Nucleotide Demand Resulting in a Targetable Metabolic Vulnerability. Cancer Cell 32: 624-638 e5

Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324: 1029-33

Vervliet T, Parys JB, Bultynck G (2016) Bcl-2 proteins and calcium signaling: complexity beneath the surface. Oncogene 35: 5079-92

Vieira AR, Abar L, Chan DSM, Vingeliene S, Polemiti E, Stevens C, Greenwood D, Norat T (2017) Foods and beverages and colorectal cancer risk: a systematic review and meta-analysis of cohort studies, an update of the evidence of the WCRF-AICR Continuous Update Project. Ann Oncol 28: 1788-1802

Volmer R, van der Ploeg K, Ron D (2013) Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. Proc Natl Acad Sci U S A 110: 4628-33

Walker GA, White TM, McColl G, Jenkins NL, Babich S, Candido EP, Johnson TE, Lithgow GJ (2001) Heat shock protein accumulation is upregulated in a long-lived mutant of Caenorhabditis elegans. J Gerontol A Biol Sci Med Sci 56: B281-7

Wang M, Kaufman RJ (2014) The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat Rev Cancer 14: 581-97

Wang W, Xie Q, Zhou X, Yao J, Zhu X, Huang P, Zhang L, Wei J, Xie H, Zhou L, Zheng S (2015) Mitofusin-2 triggers mitochondria Ca2+ influx from the endoplasmic reticulum to induce apoptosis in hepatocellular carcinoma cells. Cancer Lett 358: 47-58

Wang Y, Swiecki M, McCartney SA, Colonna M (2011) dsRNA sensors and plasmacytoid dendritic cells in host defense and autoimmunity. Immunol Rev 243: 74-90

Wei M, Fabrizio P, Hu J, Ge H, Cheng C, Li L, Longo VD (2008) Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. PLoS Genet 4: e13

Weimer S, Priebs J, Kuhlow D, Groth M, Priebe S, Mansfeld J, Merry TL, Dubuis S, Laube B, Pfeiffer AF, Schulz TJ, Guthke R, Platzer M, Zamboni N, Zarse K, Ristow M (2014) D-Glucosamine supplementation extends life span of nematodes and of ageing mice. Nat Commun 5: 3563

Williams JB, Horton BL, Zheng Y, Duan Y, Powell JD, Gajewski TF (2017) The EGR2 targets LAG-3 and 4-1BB describe and regulate dysfunctional antigen-specific CD8+ T cells in the tumor microenvironment. J Exp Med 214: 381-400

Wolfert MA, Boons GJ (2013) Adaptive immune activation: glycosylation does matter. Nat Chem Biol 9: 776-84

Woods DM, Sodre AL, Villagra A, Sarnaik A, Sotomayor EM, Weber J (2015) HDAC Inhibition Upregulates PD-1 Ligands in Melanoma and Augments Immunotherapy with PD-1 Blockade. Cancer Immunol Res 3: 1375-85

Xia X, Lei L, Qin W, Wang L, Zhang G, Hu J (2018) GCN2 controls the cellular checkpoint: potential target for regulating inflammation. Cell Death Discov 4: 20

Xia XJ, Gao YY, Zhang J, Wang L, Zhao S, Che YY, Ao CJ, Yang HJ, Wang JQ, Lei LC (2016) Autophagy mediated by arginine depletion activation of the nutrient sensor GCN2 contributes to interferon-gamma-induced malignant transformation of primary bovine mammary epithelial cells. Cell Death Discov 2: 15065

Xie H, Tang CH, Song JH, Mancuso A, Del Valle JR, Cao J, Xiang Y, Dang CV, Lan R, Sanchez DJ, Keith B, Hu CC, Simon MC (2018) IRE1alpha RNase-dependent lipid homeostasis promotes survival in Myc-transformed cancers. J Clin Invest 128: 1300-1316

Yamamori T, Meike S, Nagane M, Yasui H, Inanami O (2013) ER stress suppresses DNA double-strand break repair and sensitizes tumor cells to ionizing radiation by stimulating proteasomal degradation of Rad51. FEBS Lett 587: 3348-53

Yang H, Ma Y, Chen G, Zhou H, Yamazaki T, Klein C, Pietrocola F, Vacchelli E, Souquere S, Sauvat A, Zitvogel L, Kepp O, Kroemer G (2016) Contribution of RIP3 and MLKL to immunogenic cell death signaling in cancer chemotherapy. Oncoimmunology 5: e1149673

Yang M, Kenfield SA, Van Blarigan EL, Batista JL, Sesso HD, Ma J, Stampfer MJ, Chavarro JE (2015) Dietary patterns after prostate cancer diagnosis in relation to disease-specific and total mortality. Cancer Prev Res (Phila) 8: 545-51

Yang X, Qian K (2017) Protein O-GlcNAcylation: emerging mechanisms and functions. Nat Rev Mol Cell Biol 18: 452-465

Yang X, Zhang X, Fu ML, Weichselbaum RR, Gajewski TF, Guo Y, Fu YX (2014) Targeting the tumor microenvironment with interferon-beta bridges innate and adaptive immune responses. Cancer Cell 25: 37-48

Ye J, Kumanova M, Hart LS, Sloane K, Zhang H, De Panis DN, Bobrovnikova-Marjon E, Diehl JA, Ron D, Koumenis C (2010) The GCN2-ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation. EMBO J 29: 2082-96

Yecies JL, Manning BD (2011) Transcriptional control of cellular metabolism by mTOR signaling. Cancer Res 71: 2815-20

York A (2018) Microbiome: Gut microbiota sways response to cancer immunotherapy. Nat Rev Microbiol 16: 121

Yoshida K, Hirabayashi Y, Watanabe F, Sado T, Inoue T (2006) Caloric restriction prevents radiation-induced myeloid leukemia in C3H/HeMs mice and inversely increases incidence of tumor-free death: implications in changes in number of hemopoietic progenitor cells. Exp Hematol 34: 274-83

Yoshida K, Inoue T, Hirabayashi Y, Matsumura T, Nemoto K, Sado T (1997) Radiation-induced myeloid leukemia in mice under calorie restriction. Leukemia 11 Suppl 3: 410-2

Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, D'Agostino D, Planavsky N, Lupfer C, Kanneganti TD, Kang S, Horvath TL, Fahmy TM, Crawford PA, Biragyn A, Alnemri E, Dixit VD (2015) The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat Med 21: 263-9

Yuan A, Hsiao YJ, Chen HY, Chen HW, Ho CC, Chen YY, Liu YC, Hong TH, Yu SL, Chen JJ, Yang PC (2015) Opposite Effects of M1 and M2 Macrophage Subtypes on Lung Cancer Progression. Sci Rep 5: 14273

Zhai L, Ladomersky E, Lenzen A, Nguyen B, Patel R, Lauing KL, Wu M, Wainwright DA (2018) IDO1 in cancer: a Gemini of immune checkpoints. Cell Mol Immunol 15: 447-457

Zhang J, Pavlova NN, Thompson CB (2017) Cancer cell metabolism: the essential role of the nonessential amino acid, glutamine. EMBO J 36: 1302-1315

Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G (2003) Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 348: 203-13

Zhang L, Li Z, Ding G, La X, Yang P, Li Z (2017) GRP78 plays an integral role in tumor cell inflammation-related migration induced by M2 macrophages. Cell Signal 37: 136-148

Zhao Y, Wu T, Shao S, Shi B, Zhao Y (2016) Phenotype, development, and biological function of myeloid-derived suppressor cells. Oncoimmunology 5: e1004983

Zhou J, Mao B, Zhou Q, Ding D, Wang M, Guo P, Gao Y, Shay JW, Yuan Z, Cong YS (2014) Endoplasmic reticulum stress activates telomerase. Aging Cell 13: 197-200

Zhu L, Ploessl K, Zhou R, Mankoff D, Kung HF (2017) Metabolic Imaging of Glutamine in Cancer. J Nucl Med 58: 533-537

Zhu X, Pattenden S, Bremner R (1999) pRB is required for interferon-gamma-induction of the MHC class II abeta gene. Oncogene 18: 4940-7

Zink A, Rohrbach H, Szeimies U, Hagedorn HG, Haas CJ, Weyss C, Bachmeier B, Nerlich AG (1999) Malignant tumors in an ancient Egyptian population. Anticancer Res 19: 4273-7

Zitvogel L, Ma Y, Raoult D, Kroemer G, Gajewski TF (2018) The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies. Science 359: 1366-1370

Curriculum Vitae

INSERM U1065

JÓZEF PIOTR BOSSOWSKI

PHONE
+48 600 925 010
+33 758 293 421

EMAIL
jpbossowski@gmail.com
Jpbossowski@unice.fr

ADDRESS
Av Parc du Robiony 20
06200 NICE
France

Date of Birth: 02 April 1990
Place of Birth: Krakow
Nationality: Polish

RESEARCH

- PhD student at Jean Ehrland Ricci Team 3 — *2014/09-onwards*
  LABEX Signalife Program NICE France
  INSERM U1065
- Erasmus exchange- CNRS institute, Montpellier — *2013.02-07*
  Solange DESAGHER - Molecular Mechanisms of Apoptosis
- TEAM scholarship in Department of Immunology Joanna Cichy Labolatory – Fundation for Polish Science — *2012-2014*
- Summer practice at *Grupa Azoty S.A.* Tarnow — *2011.09*
  Department of Food Testing&Laboratory Analysis
  Department of Immunology UJ - practicant — *2010-2012*

EDUCATION

- Bachelor and Master degree in Biotechnology at the Jagiellonian University, Krakow — *2009-2014*
- Kazimierz Brodzinski General Secondary School, Tarnow — *2006-2009*
- Music school in Tarnow, I and II degree of Piano — *1997-2009*

SCHOLARSHIPS

- TEAM scholarship from Foundation for Polish Science
- Signalife – LABEX PhD program Joint, Excellent, and Dynamic Initiative project
- La Ligue 4th year PhD scholarship

PROFESSIONAL SKILLS

Laboratory skills

Mouse experimental procedures, cell culture, chemotaxis assay, IHC frozen section staining, fluorescent microscopy, PCR/qRT-PCR, (mi)RNA/DNA purification, basic sub-cloning, molecular biochemistry, ELISA, Western Blot, basic protein purification.

Conferences

- C3M seminar series presentation – Oral presentation — *04.2016*
- Journées de l'ecole Doctorale 85 de Nice – poster session — *10.2016*
- Labex SIGNALIFE Student Conference – oral session — *11.2016*
- Journées de l'ecole Doctorale 85 de Nice – prize for the best oral presentation — *09.2017*
- TOR de FRANCE international symposium– Oral presentation — *05.2018*

1. *Rubio-Patino, C., ***Bossowski, J. P.**, De Donatis, G. M., Mondragon, L., Villa, E., Aira, L. E., Chiche, J., Mhaidly, R., Lebeaupin, C., Marchetti, S., Voutetakis, K., Chatziioannou, A., Castelli, F. A., Lamourette, P., Chu-Van, E., Fenaille, F., Avril, T., Passeron, T., Patterson, J. B., Verhoeyen, E., Bailly-Maitre, B., Chevet, E. & Ricci, J. E. (2018) Low-Protein Diet Induces IRE1alpha-Dependent Anticancer Immunosurveillance, Cell Metab. 27, 828-842 e7. **IF=18.2**

2. Rubio-Patino, C., **Bossowski, J. P.,** Chevet, E. & Ricci, J. E. (2018) Reshaping the Immune Tumor Microenvironment through IRE1 signaling – In press: Trends in Molecular Medicine DOI: 10.1016/j.molmed.2018.05.005 **IF=10.7**

2. Villa, E., Proics, E., Rubio-Patino, C., Obba, S., Zunino, B., **Bossowski, J. P.**, Rozier, R. M., Chiche, J., Mondragon, L., Riley, J. S., Marchetti, S., Verhoeyen, E., Tait, S. W. G. & Ricci, J. E. (2017) Parkin-Independent Mitophagy Controls Chemotherapeutic Response in Cancer Cells, Cell Rep. 20, 2846-2859 **IF=8.3**

3. Skrzeczynska-Moncznik, J., Zabieglo, K., **Bossowski, J. P.**, Osiecka, O., Wlodarczyk, A., Kapinska-Mrowiecka, M., Kwitniewski, M., Majewski, P., Dubin, A. & Cichy, J. (2017) Eosinophils Regulate Interferon Alpha Production in Plasmacytoid Dendritic Cells Stimulated with Components of Neutrophil Extracellular Traps, J Interferon Cytokine Res. 37, 119-128. **IF=2.4**

4. Obacz, J., Avril, T., Rubio-Patino, C., **Bossowski, J. P.**, Igbaria, A., Ricci, J. E. & Chevet, E. (2017) Regulation of tumor-stroma interactions by the unfolded protein response, FEBS **IF=4.2**

5. Rubio-Patino, C., **Bossowski, J. P.**, Villa, E., Mondragon, L., Zunino, B., Proics, E., Chiche, J., Bost, F., Verhoeyen, E. & Ricci, J. E. (2016) Low carbohydrate diet prevents Mcl-1-mediated resistance to BH3-mimetics, Oncotarget. 7, 73270-73279. **IF=5.2**

6. Mojsa, B., Mora, S., **Bossowski, J. P.**, Lassot, I. & Desagher, S. (2015) Control of neuronal apoptosis by reciprocal regulation of NFATc3 and Trim17, Cell Death Differ. 22, 274-86. **IF=8.2**

7. Laura Mondragón , Rana Mhaidly ,Gian Marco De Donatis , Marie Tosselini , Pascal Dao, Antony Martin , Caroline Pons , Johanna Chiche , Marie Jacquin , Véronique Imbert , EmmaProïcs , Camila Rubio-Patiño , **Jozef P. Bossowski** , Florian Muller , Sandrine Marchetti, Elodie Villa , Jean-François Perron , Laurent Genestier , Rachid Benhida, Jean-JacquesFournié, Thierry Passeron , Jean-Ehrland Ricci and Els Verhoeyen. Submitted: GAPDH overexpression in the T cell lineage promotes angioimmunoblastic T cell lymphoma through an NF-kB dependent mechanism.

Anexes

2. Article 2 **:**

Rubio-Patino, C., **Bossowski, J. P**., Villa, E., Mondragon, L., Zunino, B., Proics, E., Chiche, J., Bost, F., Verhoeyen, E. & Ricci, J. E. (2016) Low carbohydrate diet prevents Mcl-1-mediated resistance to BH3-mimetics, Oncotarget. 7, 73270-73279.

Research Paper

Low carbohydrate diet prevents Mcl-1-mediated resistance to BH3-mimetics

Camila Rubio-Patiño[1,2], Jozef P. Bossowski[1,2], Elodie Villa[1,2], Laura Mondragón[1,2], Barbara Zunino[1,2], Emma Proïcs[1,2,3], Johanna Chiche[1,2], Frédéric Bost[2,4], Els Verhoeyen[1,2], Jean-Ehrland Ricci[1,2]

[1]Inserm, U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), Équipe "Contrôle Métabolique des Morts Cellulaires", Nice, France

[2]Université Nice Côte d'Azur, Inserm, C3M, France

[3]Centre Hospitalier Universitaire de Nice, Département d'Anesthésie Réanimation, Nice, France

[4]Inserm, U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), Équipe "Cellular and Molecular Physiopathology of Obesity and Diabetes", Nice, France

Correspondence to: Jean-Ehrland Ricci, **email:** ricci@unice.fr

Keywords: *metabolism, cancer, resistance to treatment, Mcl-1, low carbohydrate diet*

Received: *May 27, 2016* ***Accepted:*** *September 19, 2016* ***Published:*** *September 28, 2016*

ABSTRACT

Overexpression of Mcl-1 is implicated in resistance of several cancers to chemotherapeutic treatment, therefore identifying a safe way to decrease its expression in tumor cells represents a central goal. We investigated if a modulation of the diet could impact on Mcl-1 expression using a Myc-driven lymphoma model. We established that a partial reduction of caloric intake by 25% represents an efficient way to decrease Mcl-1 expression in tumor cells. Furthermore, using isocaloric custom diets, we observed that carbohydrates (CHO) are the main regulators of Mcl-1 expression within the food. Indeed, feeding lymphoma-bearing mice with a diet having 25% less carbohydrates was sufficient to decrease Mcl-1 expression by 50% in lymphoma cells. We showed that a low CHO diet resulted in AMPK activation and mTOR inhibition leading to eukaryotic elongation factor 2 (eEF2) inhibition, blocking protein translation elongation. Strikingly, a low CHO diet was sufficient to sensitize Myc-driven lymphoma-bearing mice to ABT-737-induced cell death *in vivo*. Thus reducing carbohydrate intake may represent a safe way to decrease Mcl-1 expression and to sensitize tumor cells to anti-cancer therapeutics.

INTRODUCTION

While extremely diverse in origin and in the type of associated mutations, cancer cells share common features, including the ability to use diverse sources of energy for cell proliferation and the ability to escape cell death [1]. Otto Warburg, in the 1920's, was one of the first to describe that cancer cells have a special metabolism, as they are avidly relying on glucose to produce energy [2]. This metabolic phenomenon is now referred to as the "Warburg effect". Further research established that the Warburg effect, observed in about 80% of cancers, is not only required for energy production but also for the generation of macronutrients and the redox systems that are required for the rapid proliferation of cancer cells [3]. Since this discovery, researchers and pharmaceutical companies are developing ways to modulate metabolism in order to limit tumor appearance and/or enhance treatment. One central way to control the metabolism of tumor cells is to modify the whole metabolism of the body through the modulation of food intake. Indeed, it has been known for more than a century that low caloric intake is associated with a reduced risk of several human diseases including cancer, while excess caloric intake is associated with higher cancer risk and shortened lifespan [4]. It is now clear that lowering caloric intake in general or specific macronutrients can prolong life span and improve health in a broad range of organisms when compared with unrestricted food intake. Overtime it was suggested that diet-induced regulation of insulin and its closely related hormone, Insulin-like growth factor 1 (IGF-1), were main events involved in protection against cancer incidence [4, 5]. However how food intake and how specific macronutrients impact on the response to chemotherapy is still largely unknown.

According to the International Agency for Research on Cancer (IARC), the worldwide age standardized incidence rate of non-Hodgkin lymphoma (NHL) among both sexes is estimated at 5 per 100,000 people, and is considered among the fastest rising cancer both in frequency and death rates in the United States. NHL includes a heterogeneous variety of malignancies of which the vast majority derives from B lymphocytes. Overexpression of the antiapoptotic members of the Bcl-2 family is one of the best-characterized alterations associated with NHL. Bcl-2 family members are primary regulators of mitochondrial integrity. Indeed, upon activation, Bax and Bak will induce the mitochondrial outer membrane permeabilization (MOMP) resulting in the release of inter-membrane space proteins including cytochrome c. Once released, cytochrome c will bind Apaf-1 and caspase-9 leading to apoptosome formation, caspase activation and cell death. Bax, Bak and possibility Bok are considered to be absolutely required for MOMP and for the apoptotic mitochondrial pathway as their removal leads to resistance to a variety of stimuli [6–8]. The activity of these proapoptotic proteins is tightly controlled by either the anti-apoptotic Bcl-2 family members (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, Bcl-B and A1), which restrain the induction of cell death, thus promoting cellular survival, or by the proapoptotic BH3-only proteins (Bim, Puma, Noxa, Bad, Bmf, Bid, Bik and Hrk) [9], which actively participate in inducing cell death.

Anti-apoptotic Bcl-2 family members function by directly binding BH3-only molecules as well as pro-apoptotic effectors. This notion was critical to the design of therapeutic strategies that have been developed to inhibit the prosurvival members. These include BH3 mimetics, such as ABT-737 and ABT-263 (navitoclax) [10, 11],which bind with high affinity to Bcl-2 and Bcl-xL resulting in Bax and Bak-dependent apoptosis. While efficient in some settings, the low affinity of these compounds for Mcl-1 or A1 limits their use in cells presenting high endogenous levels of Mcl-1 expression, such as aggressive forms of NHL. Mcl-1 has the particularity to have a short half-life and to be highly regulated at transcriptional, translational and post-translational levels [12]. Additionally, Mcl-1 expression is tightly associated with cell metabolism [13]. In fact, it has been shown that a shorter form of Mcl-1 could localize in the mitochondrial matrix and improve energy production [14]. Several strategies have been developed to reduce Mcl-1 expression within tumor cells. One of these is the modulation of glycolytic metabolism, which regulates Bcl-2 family members expression, especially Mcl-1, but also sensitizes tumor cells to apoptosis through the modulation of the main energy sensor of the cell, the AMPK/mTOR pathway [15–19]. We recently established that reducing the food intake of the mice by 25% (caloric restriction, CR) reduces Mcl-1 expression and sensitize lymphoma-bearing mice to BH3-mimetics [20].We therefore decided to investigate if a specific macronutrient of the food could impact on Mcl-1 expression. We fed mice bearing non Hodgkin B cell lymphoma with an isocaloric low carbohydrate (CHO) or an isocaloric low protein (PROT) diet and analyzed their response to targeted chemotherapy. Our work indicates that lowering CHO intake represents an efficient way to downregulate Mcl-1 via an AMPK/mTOR dependent control of its translation.

RESULTS

Low carbohydrate diet reduces Mcl-1 expression

Using an *in vivo* model of Myc lymphoma-mice [21], we addressed the role of caloric intake and specific nutrients in the resistance to BH3-mimetics. Myc lymphoma-bearing mice are especially well suited model to address this question. First, Eμ-*Myc* transgenic mice overexpress the c-*Myc* oncogene in the B cell lineage and develop pre-B and B-cell lymphoma with associated leukemia by several months of age [21]. Secondly, genetics and histopathology of those mice resemble human non-Hodgkin's lymphomas. Thirdly, we chose the Eμ-*Myc* model as it was largely proven to be a very robust system to characterize *in vivo* response to anti-cancer drugs [22] and to decipher the key role-played by the Bcl-2 family members in this setting [23, 24]. We therefore investigated how caloric intake may modulate the expression of this family of proteins. To this end, syngeneic C57BL/6 mice were intravenously injected with Eμ-*Myc* primary cells. Four days later, lymphoma-bearing mice were fed either *ad libitum* (control) or in CR conditions, which consists in a 25% reduction of caloric intake, in accordance with our recent work [20], we showed that a global reduction of caloric intake by 25% for 5 days was sufficient to reduce the glycemia of the mice (Figure 1A) and to decrease Mcl-1 expression by 50%(Figure 1B, 1C). On note, the mice weight was not modulated over the time of the experiment (not shown).

We then sought to determine which macronutrient was involved in the decrease of Mcl-1 protein expression. We therefore derived from the control diet two additional custom diets that contain 25% less carbohydrates (hereafter Low CHO diet) or 25% less proteins (Low PROT diet). It is worth noting that all the diets are isocaloric (see material and methods section for details). Syngeneic C57BL/6 mice were intravenously injected with Eμ-*Myc* primary cells and a few days later lymphoma-bearing mice were fed *at libitum* with one of these diets for 5 days. Of note, there was no significant difference in the weight of the mice fed with the different diets over the course of the study (not shown). We observed that only the Low CHO diet significantly reduced glycemia levels (Figure 2A). Upon sacrifice, we confirmed by flow cytometry that the CD3 and CD19 populations in the lymph nodes and

spleens were similar and therefore comparable among mice (Supplementary Figure S1A, S1B). We therefore analyzed the expression levels of the main Bcl-2 family members within the lymph nodes. Only a few days of feeding the mice with the Low CHO diet repeatedly led to a reduction of Mcl-1 expression (Figure 2B, 2C) by more than 50%. It is worth mentioning that other Bcl-2 family members analyzed (i.e. Bcl-2, Bcl-xL and Bim EL) were not significantly modulated by this diet (Figure 2B, 2C). In order to determine whether the decrease in Mcl-1

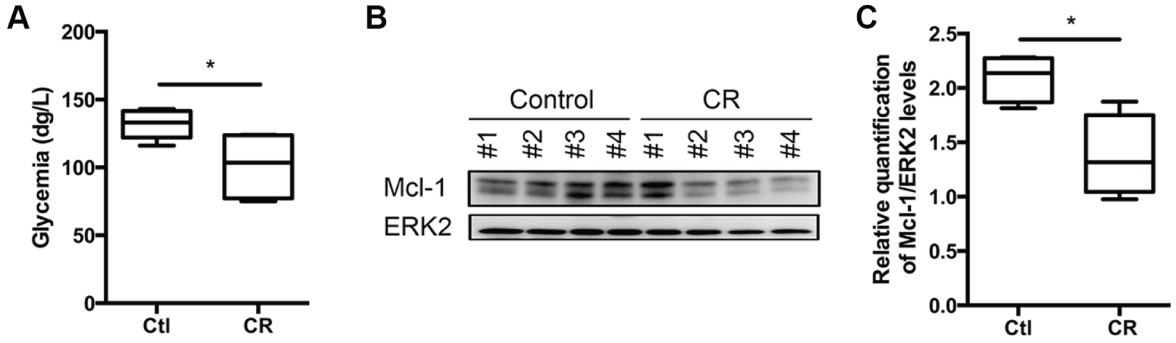


Figure 1: Caloric Restriction decreases Mcl-1 protein levels. WT C57BL/6 syngeneic mice were injected intravenously with Eμ-Myc lymphoma cells and fed ad libitum (Ctl, control) or under CR conditions for 5 days (5 mice per group). (**A**) Glycemia was measured after 5 days of dietary study. (**B**) Lymph nodes bearing lymphoma were harvested from 5 independent mice after 5 days under CR or ad libitum feeding (control) and lysates were prepared. Mcl-1 expression was analyzed by immunoblots. (**C**) Average quantification of Mcl-1 compared with ERK2 levels (used as a loading control) from samples. *$P < 0.05$.

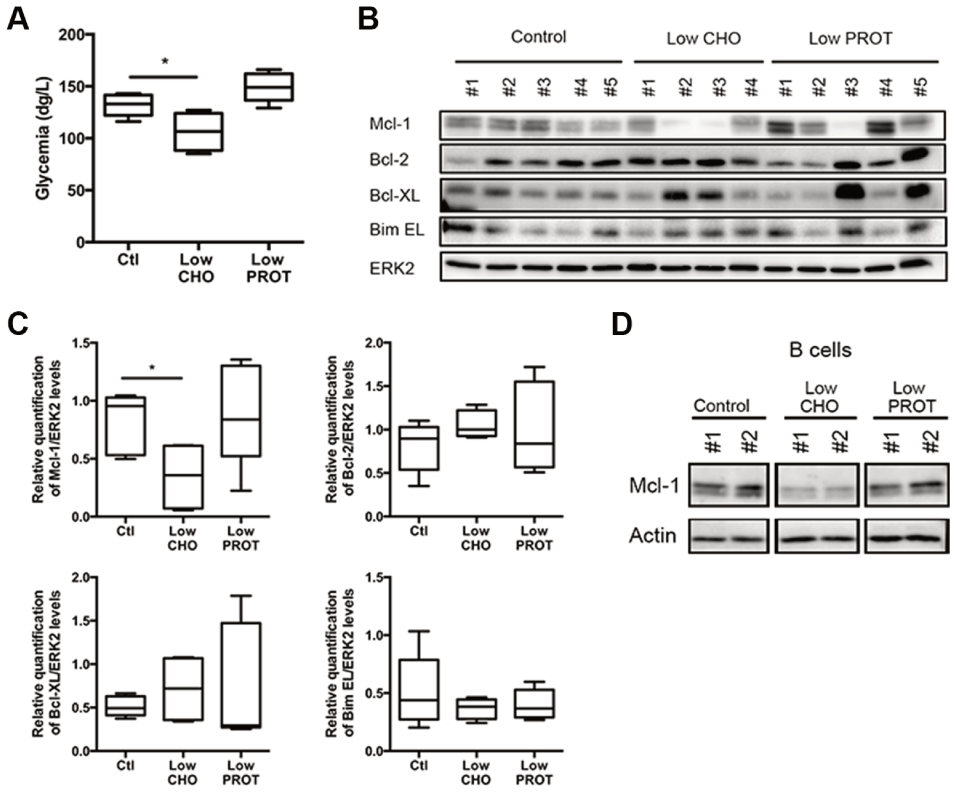


Figure 2: Low carbohydrate Intake affects Mcl-1 expression levels. WT C57BL/6 syngeneic mice were injected intravenously with Eμ-Myc lymphoma cells and fed *ad libitum* with Ctl, low CHO and low PROT diets (Ctl, $n = 5$; low CHO, $n = 4$; low PROT, $n = 5$). (**A**) Glycemia was measured after 5 days of dietary study. *$P < 0.05$. (**B**) Lymph nodes bearing lymphoma were harvested from mice after 5 days of *ad libitum* feeding with Ctl, low CHO and low PROT diets and lysates were prepared. Expression of Bcl-2 family members was analyzed by immunoblots. (**C**) Average quantification of Mcl-1, Bcl-2, Bcl-XL, and Bim EL levels compared with ERK2 levels (used as a loading control). *$P < 0.05$. (**D**) WT C57BL/6 syngeneic mice were injected intravenously with Eμ-*Myc* lymphoma cells and fed ad libitum with Ctl, low CHO and low PROT diets for 5 days. Lymph nodes bearing lymphoma were harvested from mice and B cells were sorted (Ctl, $n = 2$; low CHO, $n = 2$; low PROT, $n = 2$). Mcl-1 protein levels were analyzed by immunoblot. Actin was used as a loading control.

protein expression represents a decrease in lymphoma B cells, lysates from sorted B cells isolated from lymphoma-bearing spleens were analyzed by Western blot. B cells showed a uniformly low level of expression of Mcl-1 under Low CHO conditions, while this was not observed under control or Low PROT conditions (Figure 2D).

Altogether our data indicate that carbohydrates are the main macronutrients involved in the diet-induced reduction of Mcl-1 expression in B cells of lymphoma-bearing mice.

Diet-induced decrease of Mcl-1 occurs through a reduction of its translation in an AMPK-mTOR dependent manner

In order to determine how Mcl-1 expression was reduced upon Low CHO diet, we first measured its mRNA expression. As previously, lymphoma-bearing mice were fed with a Low CHO or a Low PROT diet for 5 days and Mcl-1 mRNA levels from the lymph nodes were analyzed by real time quantitative PCR. We did not observe any significant difference between the diets (Figure 3A), suggesting that Mcl-1 regulation upon dietary modulation occurs at the post-transcriptional level.

Among the Bcl-2 family members, Mcl-1 has the particularity of being a protein with a short half-life. We and others have reported that an inhibition of the AMPK/mTOR pathway results in the inhibition of protein translation and subsequent decrease of Mcl-1 expression [18–20, 25, 26]. It is well established that upon reduction of nutrient availability, cells will reduce protein translation, as it is one of the most ATP-demanding process in the cell. This process is mainly regulated by the AMPK/mTOR signaling pathway. We observed that AMPK was activated in the lymph nodes of lymphoma-bearing mice that were fed during 5 days with the Low CHO and Low PROT diets. We also observed an inhibition of the mTOR pathway in the lymph nodes of mice fed with the Low CHO diet as judged by the reduction in RAPTOR expression along with reduced level of phosphorylated S6, one of the mTOR targets (Figure 3B, 3C).

Eukaryotic Translation Elongation Factor 2 (eEF2) is a key regulator of protein translation that is controlled by AMPK activation and/or mTOR inhibition [27]. Indeed, its phosphorylation leads to its inactivation resulting in a block in protein translation. We observed *in vivo* that the Low CHO and the Low PROT diet resulted in the inactivation of eEF2, which was associated with a reduction in Mcl-1 expression (Figure 4A, 4B). To further investigate cap-dependent protein translation inhibition *in vivo*, we performed m^7-GTP pull down in lymphocytes of sick mice. eIF4E and associated factors were isolated with m^7-GTP (guanosine 50-triphosphate)- sepharose beads, which mimics the mRNA cap structure. As shown in Figure 4C, the amount of inhibitory 4E-BP1 associated with eIF4E increased dramatically in mice fed with a Low CHO diet, indicating an inhibition of translation rates.

Taken together, these results indicate that the Mcl-1 decrease observed *in vivo* in lymphoma-bearing mice fed with a Low CHO diet is mediated, at least in part, by the AMPK/mTOR control of its translation.

Low carbohydrate diet sensitizes lymphoma-bearing mice to ABT-737-treatment

We established that a 25% reduction in CHO was sufficient to significantly reduce Mcl-1 expression in lymphoma cells (Figures 2, 4). Eμ-*Myc* cells are known to be resistant to ABT-737 mainly because they express high Mcl-1 levels, which is poorly targeted by this BH3-mimetic. We tried to mimic the effects of macronutrient modulation *in vitro*, where Eμ-*Myc* and HeLa cells were resistant to ABT-737 but were sensitized to it when glycolysis was inhibited or glucose in the medium was modulated. *Myc*-expressing premalignant and neoplastic B cells are highly sensitive to amino acid (AA) starvation, inducing extensive cell death *in vitro* and making it hard to analyze its effect on ABT-737 resistance (Supplementary Figure S2A). In the case of HeLa cells, we observed sensitization to ABT-737 by glycolysis inhibition and modulation, but not in AA modulation and deprivation conditions. The sensitization to ABT-737 was lost when Mcl-1 was overexpressed in these cells (Supplementary Figure S2B).

We therefore investigated if the modulation of macronutrient intake was sufficient to sensitize lymphoma-bearing mice to this targeted chemotherapy. Lymphoma-bearing mice were fed *ad libitum* with the indicated diets. 3 days later, mice were intraperitoneally injected on a daily basis with 75 mg/kg ABT-737 or vehicle for 10 days (Figure 5A). At the end of the treatment, all the mice were fed with the control diet to limit the impact of those diets on tumor development. We verified that neither the type of food (Figure 5B), nor ABT-737 treatment impacted on the weight of the mice (not shown). We validated that ABT-737 treatment was effective as it led to thrombocytopenia (Figure 5C), as previously described [28]. It is worth noting that thrombocytopenia was equivalent regardless of the type of diet, consistent with Mcl-1 not playing an essential role in platelet survival [29]. Consistent with this pre-clinical model, ABT-737 treatment did not increase mice survival when animals were fed with the control diet. We observed that the different diets did not significantly modulate the overall survival of the mice in absence of chemotherapy (Ctl vs. Low CHO: $P = 0{,}3546$; Ctl vs. Low PROT: $P = 0{,}5008$; Low CHO vs. Low PROT: $P = 0{,}9596$). On the opposite, the combination of a Low CHO but not a Low PROT diet with ABT-737 doubled the overall survival of the mice (increasing the overall survival of the mice from 40 days in Low CHO conditions to 79 days in Low CHO + ABT-737 conditions, Ctl vs. Ctl+ABT-737: $P = 0{,}3179$; Low CHO vs. Low CHO+ABT-737: $P = 0{,}0487$, Low PROT vs. Low PROT+ABT-737 $P = 0{,}9187$, Figure 5D). This

effect was observed regardless of the Eμ-*Myc* clone used (See Supplementary Figure S3).

Overall, our results indicate that lowering carbohydrates but not protein intake is sufficient to reduce Mcl-1 expression and to sensitize lymphomas in mice to treatment with ABT-737.

DISCUSSION

Overexpression of Mcl-1 has been observed in several human cancers [30] and is implicated in resistance to anti-cancer therapeutics. Mcl-1 specific inhibitors have been recently developed but their use as potential therapeutic treatment is still under investigation [12]. While we obtain clinical validation of such inhibitors, several attempts to reduce Mcl-1 expression have been proposed, such as the modulation of cellular metabolism (for review [13]). Here we report on the ability of a Low CHO diet to reduce Mcl-1 protein expression and to sensitize lymphoma cells to BH3-mimetics.

While reducing food intake (caloric restriction) is a very efficient way to reduce Mcl-1 expression and to sensitize lymphoma cells to targeted therapies [20], the clinical relevance of such approach might be limited by the general condition of the patient. Indeed, cachexia that is characterized by a massive loss of total body mass, anorexia, general inflammation and pronounced muscle-wasting results in a drastic decrease of the quality of life and is associated with a poor prognosis [31]. Up to 80% of cancer patients will face cachexia and it is therefore not appropriate to reduce caloric intake in most of them. To circumvent this limitation and to benefit from the positive effects brought by CR, several options are nevertheless available. One option would be to use the so-called caloric restriction mimetics (CRm). CRm are molecules that lead to one or several physiological changes induced by CR including reduction in blood glucose, insulin, and triglycerides without modulating food intake. In this line several molecules such as metformin, resveratrol or mTOR inhibitors (rapalogues) could represent interesting options (recently reviewed in [4, 32]). We and others have described that the use of such CRm could represent a possible way to limit Mcl-1 expression *in vivo* and to sensitize tumor-bearing mice to BH3-mimetics-induced death [15–19]. However the exact mechanism on how those compounds are working at the molecular level and what are the efficient doses required *in vivo* to mimic the effects of CR in patients is still highly debated in the field [33].

Another option would be to identify which macro-component of the diet is responsible for the observed decrease of Mcl-1 upon CR in order to benefit of this effect without reducing general caloric intake. For that matter, as we established that a 25% reduction of caloric intake was sufficient to reduce Mcl-1 expression ([20]and Figure 1), we generated custom diets presenting a 25% reduction of either carbohydrates (CHO) or protein (PROT). In order to

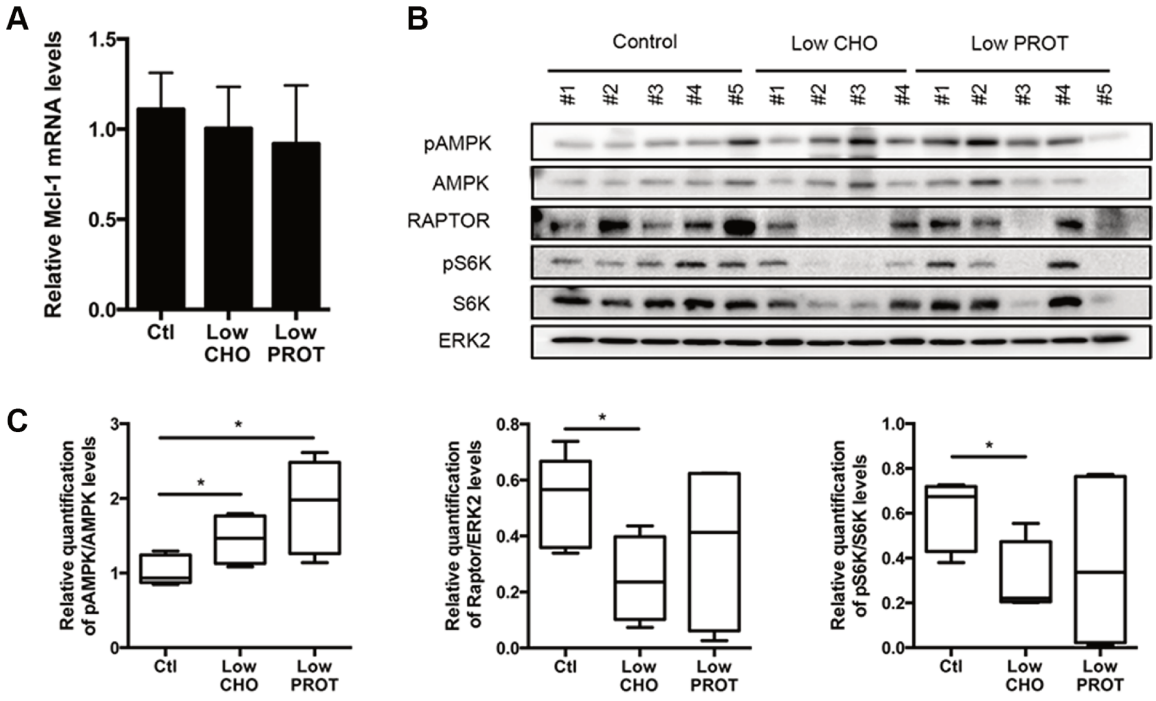


Figure 3: Low carbohydrate intake inhibits mTOR signaling. Lymph nodes bearing lymphoma were harvested after 5 days of *ad libitum* feeding with Ctl, low CHO and low PROT diets and (**A**) Mcl-1 mRNA levels were measured by real-time qPCR. (Ctl, $n = 4$; low CHO, $n = 4$; low PROT, $n = 4$) (**B**) Expression of proteins from the AMPK/mTOR pathway was analyzed by immunoblots. Average quantification of pAMPK, Raptor and pS6K compared with ERK2 levels (used as a loading control) or total protein levels. (Ctl, $n = 5$; Low CHO, $n = 4$; Low PROT, $n = 5$). *$P < 0.05$.

limit the impact of those diets on tumor development and in order to analyze the response to targeted therapy, we fed the mice with the custom diets only during the course of the treatment with ABT-737 (See Figure 5A).

B-cells, circulating and those residing in the lymph nodes, receive survival, and proliferative signals from B-cell receptor signaling through the PI3K/AKT/mTOR pathway [34]. mTOR is activated by phosphoinositide-3 kinase (PI3K)/Akt signaling in the presence of nutrients and growth factors, and inhibited by AMPK in the setting of energy deprivation. Additionally, it has been described that mTOR pathway regulates Mcl-1 by 4EBP1/mTORC1-dependent translation [19, 20, 25]. We observed that feeding lymphoma-bearing mice with a Low CHO diet resulted in AMPK activation (Figure 3), mTOR and eEF2 inhibition (Figures 3 and 4) indicating a block in protein translation. This block was further supported using m^7-GTP binding experiments (Figure 4C). As Mcl-1 has a short half-life, even a partial protein translation inhibition is sufficient to reduce its protein expression. Very importantly, we observed that the reduction in Mcl-1 expression upon a Low CHO diet was sufficient to significantly sensitize lymphoma-bearing mice to ABT-737 induced death (Figure 5D).

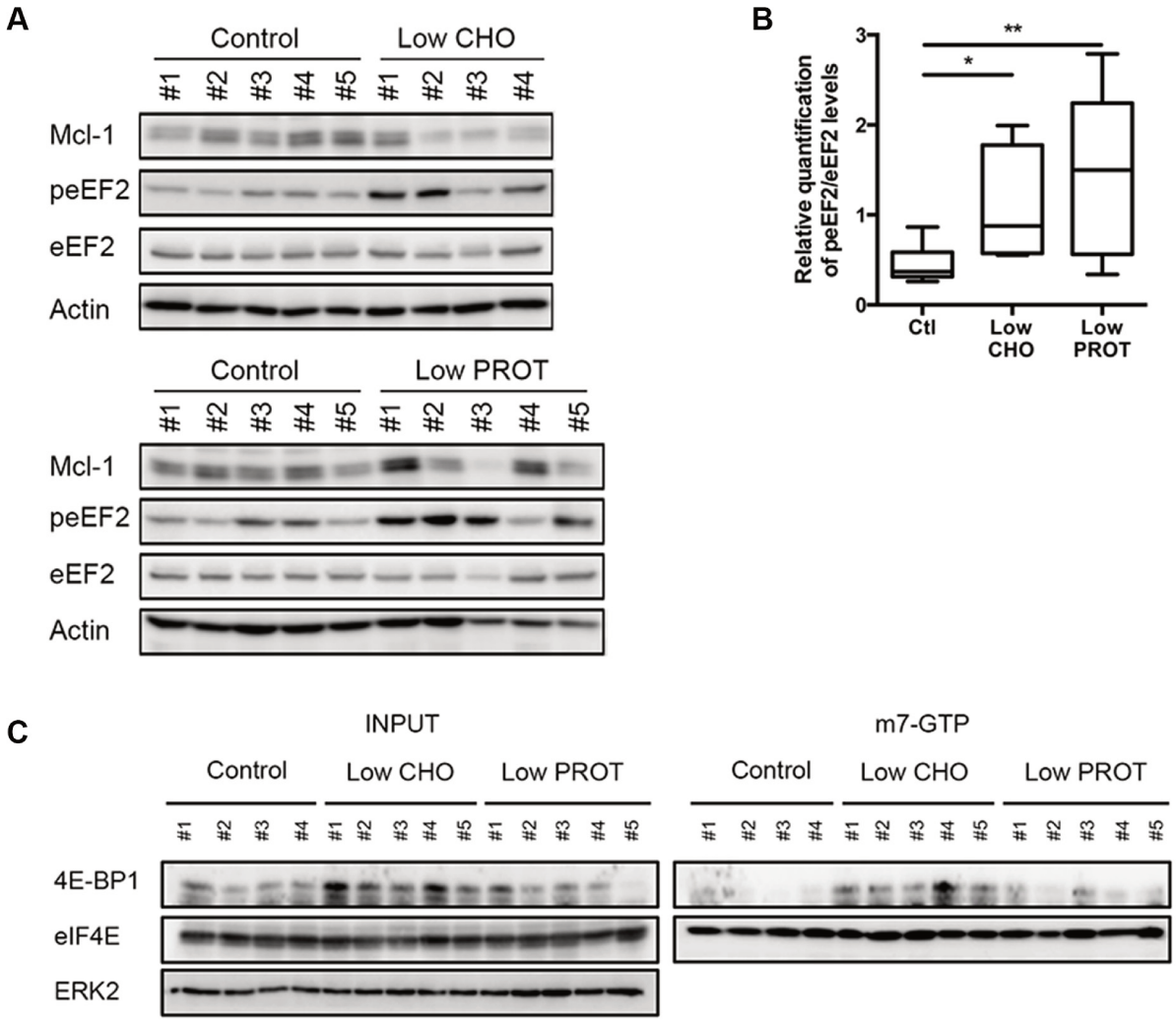


Figure 4: Low carbohydrate intake inhibits protein translation. (**A**) Lymph nodes were harvested from mice after 5 days of *ad libitum* feeding with Ctl, low CHO and low PROT diets and lysates were prepared (Ctl, $n = 5$; low CHO, $n = 4$; low PROT, $n = 5$). peEF2 expression was analyzed by immunoblot and normalized to the total protein. Actin was used as a loading control. (**B**) Average quantification of peEF2 normalized to total protein levels. (**C**) Lymphocytes were harvested from mice after 5 days of *ad libitum* feeding with Ctl, low CHO and low PROT diets and cap-dependent translation was assessed by m^7-GTP pull down. The association between 4E-BP1 and eIF4E was revealed by immunoblot. Association of the inhibitory subunit 4E-BP1 with eIF4E indicates an inhibition of translation. Expression of indicated proteins was analyzed by immunoblots. $*P < 0.05$; $**P < 0.01$.

We noticed that the effect of a Low PROT diet over Mcl-1 expression levels (Figure 2B, 2C) was very variable among experiments and not sufficient to sensitize lymphoma-bearing mice to ABT-737 treatment (Figure 5D). It has been suggested that PROT restriction primarily impacts the IGF-1 signaling pathway and that its impact on insulin levels, glycemia, ketone bodies, and free fatty acids relies on the conversion of amino acids to glucose via gluconeogenesis [35]. Therefore, changes in glucose production following a Low PROT diet are less pronounced when compared to a Low CHO diet [36]. We therefore conclude that a Low CHO diet is a more robust and efficient way to decrease Mcl-1 expression and it is very likely to be the main component, albeit not the unique involved in this effect. This conclusion is also supported by the widely described effects of glycolytic inhibitors on Mcl-1 expression and on the sensitization toward ABT-737 [4, 15–17, 20]. Altogether our results indicate that lowing circulating glucose is a key event in the decrease of Mcl-1 expression. In this sense, our results are encouraging as there are several ongoing clinical trials investigating low CHO diets in combination with conventional therapies for cancer treatment [35].

Overall, we established that a short-term reduction of carbohydrate intake represents an innovative and safe way to reduce the expression of Mcl-1, a very important oncogenic protein in lymphoma cells, thereby sensitizing them to targeted chemotherapy.

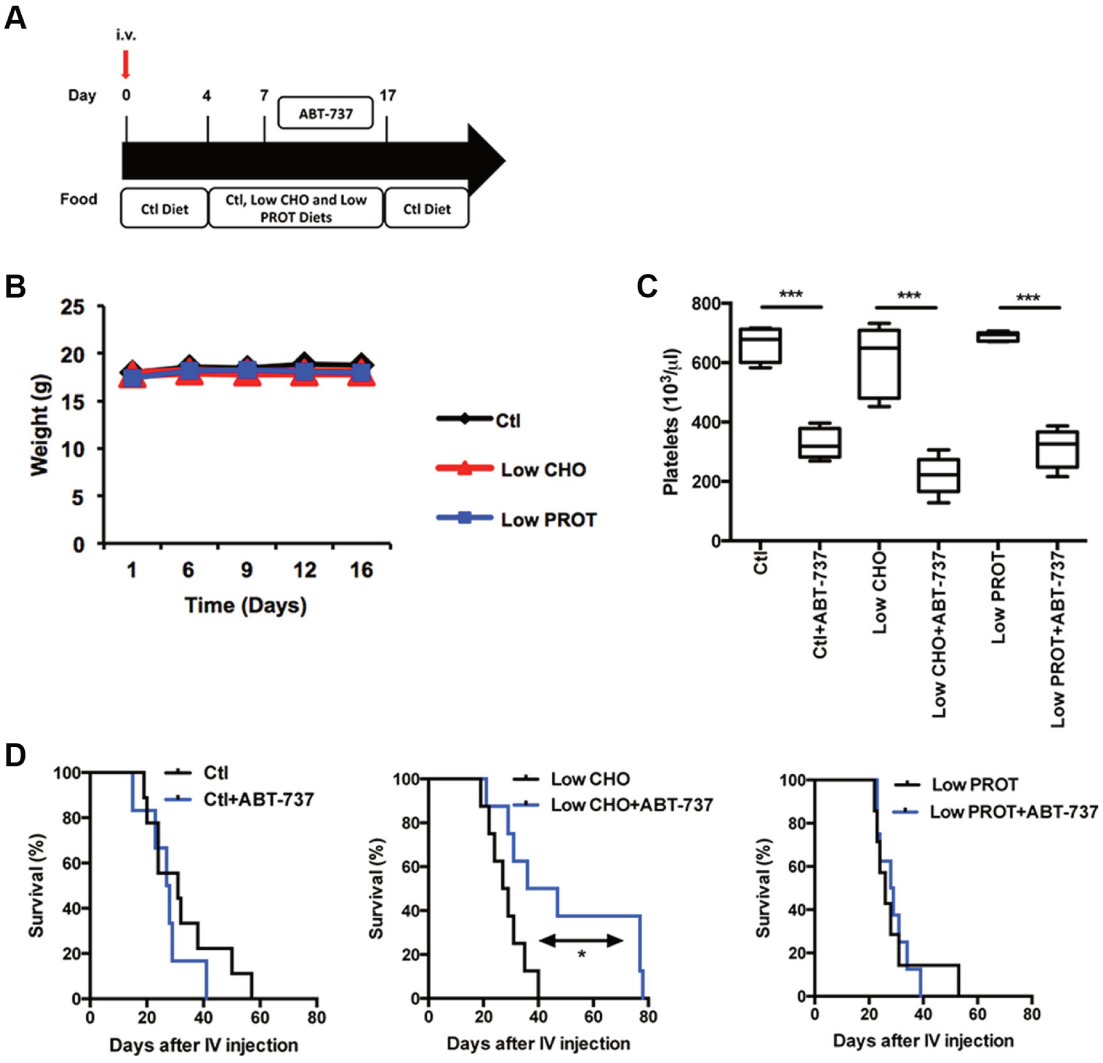


Figure 5: Low carbohydrate intake increases tumor free survival of mice treated with ABT-737. (**A**) Design of the experiment: Syngeneic C57BL/6 mice were intravenously injected with Eμ-Myc lymphoma cells and fed *ad libitum* with Ctl, low CHO and low PROT diets for 14 days. 7 days after intravenous injection, mice were treated or not for 10 days with 75 mg/kg ABT-737. Subsequently, all mice were fed ad libitum with the Ctl Diet until the time of ethical euthanasia. (**B**) Average weight of the mice that were fed for 14 days with Ctl, low CHO and low PROT diets (10 mice per group). (**C**) Numbers of platelets were measured in each group 9 days after the beginning of ABT-737 treatment (10 mice per group). (**D**) Tumor free survival of the mice are indicated for each group (Ctl $n = 9$, Ctl+ABT-737 $n = 6$, Low CHO $n = 8$, Low CHO+ABT-737 $n = 8$, Low PROT $n = 7$, Low PROT+ABT-737 $n = 8$). $^{*}P < 0.05$, $^{***}P < 0.005$. When not mentioned, differences are not significant.

Table 1: Macronutrient breakdown of diets used

Diet	Control	Low CHO	Low PROT
Carbohydrates	70,9	54,0	73,6
Proteins	19,5	26,9	14,8
Lipids	9,6	19,2	11,6

Note: Values are given in % kcal.

MATERIALS AND METHODS

CR and macronutrient modulation experiments

All animal experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee and of the regional ethics committee (approval reference PEA-232). Eμ-Myc/wild-type (WT) mice were originally obtained from the Jackson Laboratory. WT syngeneic C57BL/6 mice were intravenously (i.v.) injected with 0.1×10^6 Eμ-Myc cells and were then fed *ad libitum* with a Control, low Carbohydrates (Low CHO) or low Protein diet (Low PROT, see Table 1) custom generated by Envigo or in CR mode (75% of normal dose is 2.25 g per day per mouse). Seven days after intravenous injection of lymphoma cells, mice were intraperitoneally (I.P.) injected daily for 10 days with vehicle or ABT-737 (75 mg/kg). At the end of the ABT-737 treatment, all mice were fed *ad libitum* with control diet. Mice were monitored for lymphoma development and systemic signs of illness: apathy, breathing problems, precipitous weight loss, and limited ability to reach food or water. Animals were euthanized as soon as they showed any signs of illness. After 5 days of diet intake, glycemia was measured after a few hours of fasting by using a freestyle Optium blood glucose monitoring device. The number of Platelets was measured 1 day before the end of the ABT-737 treatment by using a Hemavet 950FS (Drew Scientific, Inc., Le Rheu, France). Mice survival was measured from the day of i.v. injection of Eμ-*Myc* mice to the death of the mice.

In vitro treatments

For nutrient modulation experiments, cells were either treated for 20 hours with 2DG (25 mM for HeLa cells and 600 μM for Eμ-*Myc* cells), cultured in a medium without glucose, with ¼ the normal concentration of amino acids or in a medium with no amino acids. In all these conditions cells were cultured in the presence or absence of 10 μM ABT-737. The empty vector and pSPEC Mcl-1 plasmid were a kind gift of Dr. Maurer (Freiburg, Germany).

Western blot analysis

Lymph nodes were collected and lysed using a Precellys 24 homogenizer (3×30 s, $6500 \times g$) in buffer A :10 mM HEPES (pH 7.4), 150 mM NaCl, 5 mM EDTA, 1% NP40, 10 μg/mL aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM leupeptin. CD19+ cells were isolated from the spleens of lymphoma-bearing mice using autoMACS (Miltenyi Biotec). CD19+ sorted cells and other cell lines were lysed in in Laemmli sample buffer. Proteins were immunoblotted with the indicated antibodies. Immunoblots were revealed (FUJIFILM LAS4000, France) using enhanced chemiluminescence detection kit (Pierce) and quantification was made using ImageJ software. The antibody Anti Mcl-1 was obtained from Rockland (Gilbertsville, PA, USA). Anti Bcl-xL, Bcl-2, Bim, pAMPK (Thr172), AMPK, RAPTOR, pS6K(Thr389), S6K, peEF2 (Thr56), eEF2, 4E-BP1, eIF4E and were purchased from Cell Signaling Technology® (Danvers, MA, USA). Anti-ERK2 and Actin were purchased from Santa Cruz (Santa Cruz, CA, USA).

Quantitative reverse transcription-PCR analysis

Total RNA was isolated from lymphoma cells using the RNeasy Micro Kit (Qiagen, Paris, France) according to the manufacturer's protocol. After reverse transcription-PCR, the relative mRNA expression level of mouse Mcl-1 was obtained by real-time quantification PCR, using the TaqMan PCR Master Mix (Eurogentec, Seraing, Belgium) and TaqMan assay primer set (Applied Biosystems, Foster City, CA, USA) on the 7500 Fast and the Step One (Applied Biosystems) according to the manufacturer's instructions. The housekeeping gene RPLP0 was used as a control for RNA quality, and used for normalization.

7-Methyl-GTP cap binding assay

Spleens from mice fed *ad libitum* with a Control, Low CHO or Low PROT diet were lysed in 400 μl of 20 mM Tris pH 7.5, 100 mM KCl, 20 mM β-glycerophosphate, 1mm dithiothreitol, 250 μM Na_3VO_4, 10 mM NaF, 1 mM EDTA, 1 mM ethylene glycol tetraacetic acid, 1 mM phenylmethylsulfonyl fluoride and lysed using a Precellys 24 homogenizer (2×30 s, $6500 \times g$). After centrifugation (13000 g—10 min—4°C), 450 μg of protein was applied to 50 μl of m^7-GTP-sepharose 4B beads (Jena Bioscience, Jena, Germany) and incubated for 2 hours at 4°C. The beads were washed and then boiled in Laemmli sample buffer. After SDS–PAGE resolution, the association of 4E-BP1 with eIF4E was detected by western blot.

Cell viability and flow cytometry

Flow Cytometry (MACS-Quant Analyzer Miltenyi Biotec) was used to analyze cell viability by looking at plasma membrane permeabilization of cells using 4′,6-diamidino-2-phenylindole (DAPI) staining. Cell viability was measured as the percentage of DAPI-negative cell population. Lymphocyte subpopulations from lymphoma-bearing lymph nodes and spleens were assessed by flow cytometry using antibodies against CD3 and CD19 (BD Bioscience, Franklin Lakes, NJ, USA).

Statistics

The data are expressed as mean ± standard deviation. Differences in calculated means between groups were assessed by two-sided Student *t* tests. For experiments involving more than two groups, differences in the calculated mean values between the groups were assessed by one-way analysis of variance, followed by a Fisher test, and in cases in which significant differences were detected, a Tukey honestly significant difference test was used. Kaplan-Meier survival analyses were performed, and survival curves were compared by using log-rank tests. For *in vitro* experiments, differences in the calculated mean values (binomial law) between the groups were assessed by two-way analysis of variance followed by a χ^2 test. A P value less than 0.05 was considered significant.

ACKNOWLEDGMENTS

We gratefully acknowledge the Centre Méditerranéen de Médecine Moléculaire animal facility.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

GRANT SUPPORT

This work was supported by the Fondation ARC (Association pour la Recherche sur le Cancer) and the Agence Nationale de la Recherche (LABEX SIGNALIFE ANR-11-LABX-0028-01). C.R.P. is supported by the Fondation ARC. J.C. by foundation Tourre, L.M by Fondation pour la Recherche Médicale. FB is a CNRS investigator.

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646–674.
2. Munoz-Pinedo C, El Mjiyad N, Ricci JE. Cancer metabolism: current perspectives and future directions. Cell Death Dis. 2012; 3:e248.
3. Mayers JR, Vander Heiden MG. Famine versus feast: understanding the metabolism of tumors *in vivo*. Trends Biochem Sci. 2015; 40:130–140.
4. Meynet O, Ricci JE. Caloric restriction and cancer: molecular mechanisms and clinical implications. Trends in molecular medicine. 2014.
5. Klement RJ, Fink MK. Dietary and pharmacological modification of the insulin/IGF-1 system: exploiting the full repertoire against cancer. Oncogenesis. 2016; 5:e193.
6. Lindsten T, Ross AJ, King A, Zong WX, Rathmell JC, Shiels HA, Ulrich E, Waymire KG, Mahar P, Frauwirth K, Chen Y, Wei M, Eng VM, et al. The combined functions of proapoptotic Bcl-2 family members bak and bax are essential for normal development of multiple tissues. Mol Cell. 2000; 6:1389–1399.
7. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB, Korsmeyer SJ. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science. 2001; 292:727–730.
8. Llambi F, Wang YM, Victor B, Yang M, Schneider DM, Gingras S, Parsons MJ, Zheng JH, Brown SA, Pelletier S, Moldoveanu T, Chen T, Green DR. BOK Is a Non-canonical BCL-2 Family Effector of Apoptosis Regulated by ER-Associated Degradation. Cell. 2016; 165:421–433.
9. Llambi F, Green DR. Apoptosis and oncogenesis: give and take in the BCL-2 family. Curr Opin Genet Dev. 2011; 21:12–20.
10. Deng J, Carlson N, Takeyama K, Dal Cin P, Shipp M, Letai A. BH3 profiling identifies three distinct classes of apoptotic blocks to predict response to ABT-737 and conventional chemotherapeutic agents. Cancer Cell. 2007; 12:171–185.
11. Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Marsh KC, Mitten MJ, Nimmer P, Roberts L, Tahir SK, Xiao Y, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res. 2008; 68:3421–3428.
12. Beekman AM, Howell LA. Small-Molecule and Peptide Inhibitors of the Pro-Survival Protein Mcl-1. ChemMedChem. 2016; 11:802–813.
13. Villa E, Ricci JE. How does metabolism affect cell death in cancer? FEBS J. 2015.
14. Perciavalle RM, Stewart DP, Koss B, Lynch J, Milasta S, Bathina M, Temirov J, Cleland MM, Pelletier S, Schuetz JD, Youle RJ, Green DR, Opferman JT. Anti-apoptotic MCL-1 localizes to the mitochondrial matrix and couples mitochondrial fusion to respiration. Nature cell biology. 2012; 14:575–583.
15. Zagorodna O, Martin SM, Rutkowski DT, Kuwana T, Spitz DR, Knudson CM. 2-deoxyglucose-induced toxicity is regulated by Bcl-2 family members and is enhanced by antagonizing Bcl-2 in lymphoma cell lines. Oncogene. 2012; 31:2738–2749.

16. Levesley J, Steele L, Taylor C, Sinha P, Lawler SE. ABT-263 enhances sensitivity to metformin and 2-deoxyglucose in pediatric glioma by promoting apoptotic cell death. PLoS One. 2013; 8:e64051.

17. Muhlenberg T, Grunewald S, Treckmann J, Podleska L, Schuler M, Fletcher JA, Bauer S. Inhibition of KIT-glycosylation by 2-deoxyglucose abrogates KIT-signaling and combination with ABT-263 synergistically induces apoptosis in gastrointestinal stromal tumor. PLoS One. 2015; 10:e0120531.

18. Meynet O, Beneteau M, Jacquin MA, Pradelli LA, Cornille A, Carles M, Ricci JE. Glycolysis inhibition targets Mcl-1 to restore sensitivity of lymphoma cells to ABT-737-induced apoptosis. Leukemia. 2012; 26:1145–1147.

19. Pradelli LA, Beneteau M, Chauvin C, Jacquin MA, Marchetti S, Munoz-Pinedo C, Auberger P, Pende M, Ricci JE Glycolysis inhibition sensitizes tumor cells to death receptors-induced apoptosis by AMP kinase activation leading to Mcl-1 block in translation. Oncogene. 2010; 29:1641–1652.

20. Meynet O, Zunino B, Happo L, Pradelli LA, Chiche J, Jacquin MA, Mondragon L, Tanti JF, Taillan B, Garnier G, Reverso-Meinietti J, Mounier N, Michiels JF, et al. Caloric restriction modulates Mcl-1 expression and sensitizes lymphomas to BH3 mimetic in mice. Blood. 2013; 122:2402–2411.

21. Harris AW, Pinkert CA, Crawford M, Langdon WY, Brinster RL, Adams JM. The E mu-myc transgenic mouse. A model for high-incidence spontaneous lymphoma and leukemia of early B cells. The Journal of experimental medicine. 1988; 167:353–371.

22. Schmitt CA, Rosenthal CT, Lowe SW. Genetic analysis of chemoresistance in primary murine lymphomas. Nat Med. 2000; 6:1029–1035.

23. Happo L, Cragg MS, Phipson B, Haga JM, Jansen ES, Herold MJ, Dewson G, Michalak EM, Vandenberg CJ, Smyth GK, Strasser A, Cory S, Scott CL. Maximal killing of lymphoma cells by DNA damage-inducing therapy requires not only the p53 targets Puma and Noxa, but also Bim. Blood. 2010; 116:5256–5267.

24. Grabow S, Delbridge AR, Aubrey BJ, Vandenberg CJ, Strasser A. Loss of a Single Mcl-1 Allele Inhibits MYC-Driven Lymphomagenesis by Sensitizing Pro-B Cells to Apoptosis. Cell Rep. 2016; 14:2337–2347.

25. Mills JR, Hippo Y, Robert F, Chen SM, Malina A, Lin CJ, Trojahn U, Wendel HG, Charest A, Bronson RT, Kogan SC, Nadon R, Housman DE, et al. mTORC1 promotes survival through translational control of Mcl-1. Proc Natl Acad Sci USA. 2008; 105:10853–10858.

26. Coloff JL, Macintyre AN, Nichols AG, Liu T, Gallo CA, Plas DR, Rathmell JC. Akt-Dependent Glucose Metabolism Promotes Mcl-1 Synthesis to Maintain Cell Survival and Resistance to Bcl-2 Inhibition. Cancer Res. 2011; 71:5204–5213.

27. Leprivier G, Remke M, Rotblat B, Dubuc A, Mateo AR, Kool M, Agnihotri S, El-Naggar A, Yu B, Prakash Somasekharan S, Faubert B, Bridon G, Tognon CE, et al. The eEF2 Kinase Confers Resistance to Nutrient Deprivation by Blocking Translation Elongation. Cell. 2013; 153:1064–1079.

28. Zhang H, Nimmer PM, Tahir SK, Chen J, Fryer RM, Hahn KR, Iciek LA, Morgan SJ, Nasarre MC, Nelson R, Preusser LC, Reinhart GA, Smith ML, et al. Bcl-2 family proteins are essential for platelet survival. Cell Death Differ. 2007; 14:943–951.

29. Debrincat MA, Josefsson EC, James C, Henley KJ, Ellis S, Lebois M, Betterman KL, Lane RM, Rogers KL, White MJ, Roberts AW, Harvey NL, Metcalf D, et al. Mcl-1 and Bcl-x(L) coordinately regulate megakaryocyte survival. Blood. 2012; 119:5850–5858.

30. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, Barretina J, Boehm JS, Dobson J, Urashima M, Mc Henry KT, Pinchback RM, Ligon AH, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010; 463:899–905.

31. Porporato PE. Understanding cachexia as a cancer metabolism syndrome. Oncogenesis. 2016; 5:e200.

32. Bost F, Decoux-Poullot AG, Tanti JF, Clavel S. Energy disruptors: rising stars in anticancer therapy? Oncogenesis. 2016; 5:e188.

33. Chandel NS, Avizonis D, Reczek CR, Weinberg SE, Menz S, Neuhaus R, Christian S, Haegebarth A, Algire C, Pollak M. Are Metformin Doses Used in Murine Cancer Models Clinically Relevant? Cell metabolism. 2016; 23:569–570.

34. Choudhary GS, Al-Harbi S, Mazumder S, Hill BT, Smith MR, Bodo J, Hsi ED, Almasan A. MCL-1 and BCL-xL-dependent resistance to the BCL-2 inhibitor ABT-199 can be overcome by preventing PI3K/AKT/mTOR activation in lymphoid malignancies. Cell Death Dis. 2015; 6:e1593.

35. Klement RJ. Mimicking caloric restriction: what about macronutrient manipulation? A response to Meynet and Ricci. Trends in molecular medicine. 2014; 20:471–472.

36. Westman EC, Mavropoulos J, Yancy WS, Volek JS. A review of low-carbohydrate ketogenic diets. Curr Atheroscler Rep. 2003; 5:476–483.

3. Article 3:

Rubio-Patino, C., **J. P. Bossowski**, E. Chevet and J. E. Ricci (2018). "Reshaping the Immune Tumor Microenvironment Through IRE1 Signaling." Trends Mol Med 24(7): 607-614.

Trends in Molecular Medicine

CellPress
REVIEWS

Opinion

Reshaping the Immune Tumor Microenvironment Through IRE1 Signaling

Camila Rubio-Patiño,[1] Jozef P. Bossowski,[1] Eric Chevet,[2,3] and Jean-Ehrland Ricci[1,*]

The ability of a tumor cell to cope with environmental and intracellular stress depends on its capacity to activate appropriate adaptive pathways. As such, the endoplasmic reticulum (ER) adjusts the adaptive capacity of tumor cells by engaging the unfolded protein response (UPR). The UPR maintains the functionality of the secretory pathway, thereby allowing tumor cells to shape their microenvironment, thus likely determining the nature of the tumor immune response. Consequently, this makes the UPR very relevant in the context of cancer therapeutics. We focus here on inositol-requiring enzyme 1α (IRE1) and compile novel molecular mechanisms demonstrating that tumoral UPR controls the tumor microenvironment (TME) and the immune response, therefore opening potential novel therapeutic avenues.

Highlights

ER stress and the UPR strongly affect tumor progression *in vivo* and *in vitro*.

The UPR is a major regulator of inflammation, cell death, angiogenesis, and metabolism. In addition, it can affect cancer cell immune recognition through processes that are yet not fully understood.

The IRE1 branch of the UPR is so far the best-documented in its ability to control cell death or survival in tumors.

An increase in IRE1 signaling has been associated with an increase in mRNA expression for key antitumor T cell markers in tumors from colorectal, glioblastoma, and cancer melanoma patients.

The UPR: Controlling the Brake of Cancer

The ability of cancer cells to respond to extrinsic and intrinsic stress depends on their capacity to successfully activate appropriate adaptive pathways [1,2]. In the course of carcinogenesis, intrinsic (e.g., oncogene-driven protein synthesis, reactive oxygen species) or extrinsic (e.g., hypoxia, nutrient shortage, chemotherapy) challenges impinge on cellular protein homeostasis (**proteostasis**; see Glossary) [3]. In the **endoplasmic reticulum** (ER), an imbalance in proteostasis leads to a situation known as ER stress [2,4]. To restore ER proteostasis an adaptive signaling pathway, the UPR, is triggered [3] that is mainly controlled by three ER-resident sensors: IRE1, protein kinase RNA-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) (Figure 1 and Box 1). The UPR attenuates protein translation, enhances protein folding and quality control, and increases ER clearance capacity. As such, UPR-mediated adaptation has been proposed to contribute to cancer development [5]. This was later confirmed in various cancers and was found to play either protumorigenic [6] or antitumorigenic roles [7,8].

The Dual Role of the IRE1 Signaling in Cancer Cell Fate: Life or Death

The UPR can affect tumor cell biology either as a barrier to tumor development or by promoting established tumors (Figure 1). The IRE1 branch of the UPR has been so far the best-documented in its ability to control cell death or survival in tumors [9,10]. IRE1 signals

Box 1. The ER and the UPR

The UPR is predominantly an adaptive pathway, but when ER stress cannot be resolved the terminal UPR is triggered to promote cell death, typically by apoptosis [12]. Notably, cancer cells generally display higher basal ER stress than their non-tumoral counterparts, and they modulate the UPR to fuel growth and survival [39]. The different UPR branches not only determine cell fate but can also shape the TME in part by controlling the secretory pathway [29]. By reprogramming the cells and adjusting the secretory pathway, UPR-dependent extracellular signals can modulate the host stroma, including immune cells. This makes the UPR an appealing candidate mechanism by which one could harness the host immune system to combat cancer.

[1]Université Côte d'Azur, Institut National de la Santé et de la Recherche Médicale (INSERM) Centre Méditerranéen de Médecine Moléculaire (C3 M), Nice, France
[2]INSERM Unité 1242 Chemistry, Oncogenesis, Stress, Signaling, Université Rennes 1, Rennes, France
[3]Centre de Lutte Contre le Cancer Eugène Marquis, Rennes, France

*Correspondence:
ricci@unice.fr (J.-E. Ricci).

are predominantly mediated by its RNase activity through either the non-conventional splicing of X-box binding protein 1 (s*XBP1*) mRNA or **regulated IRE1-dependent decay of RNA** (RIDD). IRE1 adaptive signals are mainly mediated by the transcription factor sXBP1 [9]. Alternatively, RIDD can either promote survival through the degradation of RNA coding for secretory or transmembrane proteins, thereby reducing the ER load [11], or can promote cell death through the degradation of mRNA encoding antiapoptotic proteins and secretory proteins involved in protein folding [1]. Interestingly, it has been shown that IRE1 can recruit TNF receptor-associated factor-2 (TRAF2) and activate c-Jun N-terminal kinase (JNK) which regulates apoptotic pathways by, for example, phosphorylating the pro-apoptotic protein BIM and inhibiting BCL-2 [12,13]. IRE1 has also been shown to facilitate tumor growth *in vivo* by promoting the expression of the cancer-driver β-catenin, and its inhibition or knockdown impairs colonic tumorigenesis in an immunodeficient mouse model [14]. Altogether, IRE1 controls life and death decision mechanisms under stressful conditions.

IRE1 Signaling Host Immune Cells

In vivo, tumor progression or regression not only depends on activation of the UPR in tumor cells but can also be controlled by UPR induction in stromal cells, including immune cells [2,15]. Tumor-associated dendritic cells (DCs) isolated from mice and human ovarian tumors exhibit robust sXBP1 activation compared to DCs from naive hosts. This turns out to be immunosuppressive because it promotes tumor progression and **metastasis** by impairing T cell activation [15]. Moreover, XBP1 depletion leads to hyperactivation of IRE1 and downstream RIDD, as shown in mice deficient for XBP1 in DCs [16]. This compensatory RIDD induction results in defects in cross-presentation of dead cell-derived antigens by $CD8\alpha^+$ DCs [11,16]. Nevertheless, RIDD activity in DCs has only been reported in the context of experimental *XBP1* deletion, and a physiological role for RIDD has yet to be demonstrated in the different DC populations. Another example is observed in polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), which are regulators of the immune response in cancer patients and have been shown to promote tumor progression. Indeed, in these cells IRE1 controls the conversion of neutrophils into PMN-MDSCs. PMN-MDSCs isolated from non-small cell lung cancer, head and neck cancer, colon cancer, and multiple myeloma patients expressed high levels of lectin-type oxidized LDL receptor-1 (LOX-1) compared to neutrophils isolated from the same patients. LOX-1 expression depends on sXBP1 [17]. sXBP1 induction by thapsigargin in neutrophils from healthy patients increased LOX-1 expression, which converted these cells to immunosuppressive PMN-MDSCs [17]. In addition to its immunosuppressive functions, the IRE1/XBP1 pathway was also shown in several mouse models to be important for the differentiation of immature progenitors into plasma B cells, DCs, and antigen-specific effector $CD8^+$ T cells [16,18–20].

What happens when cancer cells are immune cells? Do the same dualistic pathways govern cell fate? In this case, XBP1-dependent naïve cell differentiation into plasma B cells and effector T cells induced by fasting can block the development of acute lymphoblastic leukemia in a human xenograft model in sublethally irradiated severe combined immunodeficiency (SCID) recipient mice [20]. Alternatively, impairment of the IRE1/XBP1 pathway was identified as a hallmark of germinal center B cell diffuse large B cell lymphoma, and doxycycline-induced XBP1 protein expression reduced tumor growth in an immunocompromised AGR129 mouse xenograft model [21]. Notably, constitutive expression of sXBP1 in murine B cells promotes a disease in mice that resembles multiple myeloma [22], indicating that the IRE1 pathway can be tumor-promoting for some immune cancer cell types but can negatively impact on tumor growth in others. Once again, this raises the question of whether the effect of

Glossary

Angiogenesis: the formation of new blood vessels that fuel cancer cells with oxygen and nutrients.
Autophagy: an intracellular degradation process that takes place via the delivery of cytoplasmic entities to the lysosomes, where macromolecules are lysed and their components recycled.
Damage-associated molecular patterns (DAMPs): intracellular molecules that function as 'eat me' signals for the immune system when exposed by or released from the cell; these promote and amplify the immune response.
Endoplasmic reticulum (ER): a highly organized organelle with diverse functions, including lipid production, calcium homeostasis, drug detoxification, and protein synthesis. The ER is equipped with the biochemical machinery to promote proper protein maturation and folding, assess protein quality, and direct defective proteins to repair or degradation processes.
ER-associated protein degradation (ERAD): a pathway directing misfolded proteins in the ER for ubiquitination and proteasomal degradation.
Immunogenic dell death (ICD): a type of cell death that triggers an immune response via the release of DAMPS. Most chemotherapies used in the clinic induce tolerogenic cancer cell death, a 'silent' type of death that is not recognized by the immune system.
Immunosurveillance: the processes by which the host immune system recognizes and targets cancer cells.
Macronutrient modulation: varying the ratio of the macronutrients in the diet to meet specific metabolic needs.
Metastasis: the multistep process of cancer cell migration from its primary tumor site leading to colonization of remote tissues.
Proteostasis: maintenance of proper protein functioning in the cell.
Regulated IRE1-dependent decay of RNA (RIDD): the mechanism of microRNA/mRNA degradation via the endoRNase activity of activated IRE1.
Transmissible ER stress (TERS): cell contact-independent

activation of the pathway on tumor progression depends on the balance between the activation of pathways downstream from IRE1.

transmission of ER stress between cancer cells and from cancer cells to cells of TME.
Tumor microenvironment (TME): the unique environment consisting of cancer and non-cancer (stromal, immune) cells present in tumors that is characterized by hypoxia, nutrient deprivation, and reduced pH.

When Tumor Cells Use IRE1 Signals To Communicate with the Immune System

Modulation of ER stress in immune cells is not the only way for the UPR to regulate the immune response. The induction of the different arms of the UPR in tumor cells can result in crosstalk between the tumor and the immune system. The UPR-dependent regulation of **damage-associated molecular patterns** (DAMPs), cytokines (Box 2), and **transmissible ER stress** (TERS, Box 3), among other signals, can result in **immunosurveillance** or immune system evasion (Figure 1). However, the notion that the UPR can regulate the anticancer immune response is recent because previous cancer research has mostly been carried out *in vitro* or in immunodeficient mouse models. Notably, new evidence supports the concept of a dual role of the UPR in tumor–immune system crosstalk, thereby yielding either immunosuppressive or immunogenic outcomes. This makes the interaction between tumor cells and the **tumor microenvironment** (TME) even more complex.

Tumor IRE1 downstream pathways can carry different messages when communicating with the immune system, and a dual role for sXBP1 and RIDD has been established. In glioblastoma, high and low IRE1 activity tumor classification revealed that patients bearing tumors with a $sXBP1^{low}/RIDD^{high}$ signature showed better survival than those with a $sXBP1^{high}/RIDD^{low}$ signature [23]. In the same study, sXBP1 was found to control protumorigenic signals that promote macrophage recruitment to the tumor and **angiogenesis**, while RIDD dampened angiogenesis and cell migration. The finding that RIDD may exert antitumoral effects is supported by recent results obtained in conditions of **macronutrient modulation**. In cancer mouse models of lymphoma, and in xenograft models of colorectal cancer and melanoma, a moderate reduction of protein intake resulted in the induction of the IRE1/RIDD–RIG-I pathway in tumor cells. When IRE1 is activated, it cleaves mRNAs targeted to the ER and produces fragments that resemble those of pathogens (lack of 5′ caps or 3′ polyA tails), thus activating retinoic acid-inducible gene I (RIG-I) [24,25]. This gives rise to an anticancer CD8-dependent immune response that was not observed when mice were fed a control diet [25]. Using a gene signature reflecting IRE1 activation, tumor stratification into high and low IRE1 activity groups showed an association between an increase in IRE1 signaling and an increase in mRNA expression of key antitumor T cell markers in tumors from colorectal cancer, glioblastoma, and melanoma patients. Accordingly, RIG-I was previously proposed to be a tumor suppressor in patients with hepatocellular carcinoma [26], and its activation induces the secretion of extracellular vesicles by human and mouse melanoma cells *in vitro* that can induce natural killer (NK) cell-mediated lysis of melanoma cells *ex vivo* [27]. Furthermore, murine pancreatic cancer cells treated with RIG-I-like helicase ligands undergo **immunogenic cell death** (ICD) associated with calreticulin (CRT) exposure and the release of HMGB1 (high mobility group box 1). This results in activation of DCs in *ex vivo* coculture experiments [28].

Another way of regulating tumor/immune system crosstalk is to modulate the tumor cell secretome through the direct control of cytokine production by the UPR at the transcriptional and post-transcriptional levels (Box 2) [29,30] or through TERS [31]. TERS molecules are released by cancer cells undergoing ER stress and UPR activation (Box 3). Proinflammatory and anti-inflammatory cytokines have a direct impact on tumor progression because they control and alter the TME. There is autocrine and endocrine regulatory feedback between the UPR and cytokines, meaning that the UPR can induce cytokine production and vice versa.

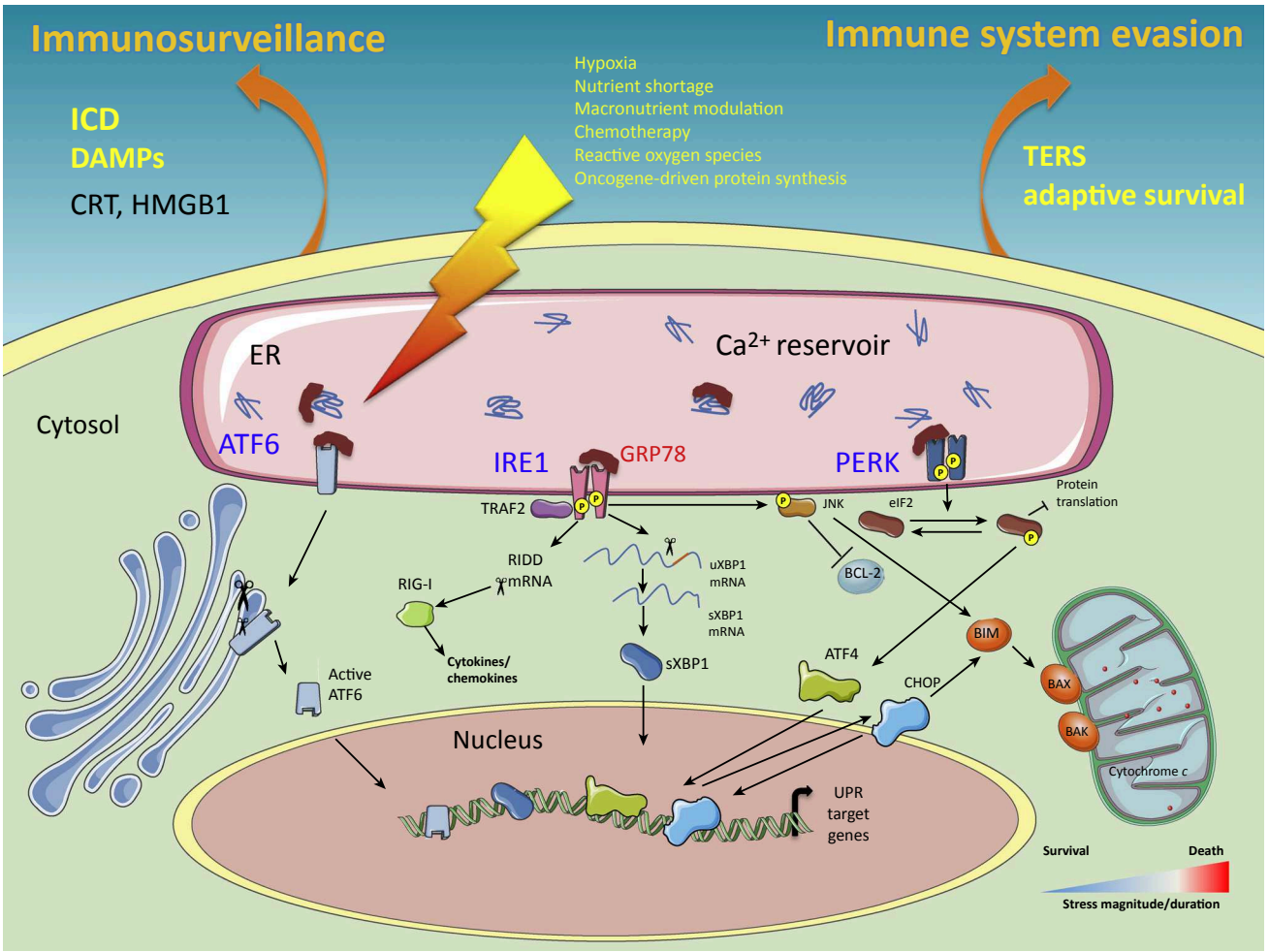


Figure 1. Unfolded Protein Response (UPR) Signaling. The UPR is primarily an adaptive response that is transduced by three endoplasmic reticulum (ER)-resident transmembrane stress sensors, namely inositol-requiring enzyme 1α (hereafter referred to as IRE1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6). These stress sensors are kept inactive through binding to the ER-resident HSP70 family chaperone GRP78 (also known as BiP). Upon stress (hypoxia, nutrient shortage, macronutrient modulation, chemotherapy, reactive oxygen species, oncogene-driven protein synthesis, among others) and accumulation of misfolded proteins in the ER, GRP78 dissociates from the sensors, thereby allowing their activation [1]. IRE1 is the most evolutionarily conserved UPR sensor and harbors both serine/threonine kinase and endoRNase catalytic domains in its cytosolic portion. Upon GRP78 dissociation, IRE1 undergoes oligomerization and *trans*-autophosphorylation, enabling its RNase activity. IRE1 RNase catalyzes the non-conventional splicing of *XBP1* mRNA, thus yielding an open reading frame shift. The spliced *XBP1* translation product, sXBP1, is a potent transcription factor that targets genes encoding proteins participating in protein folding, **ER-associated protein degradation** (ERAD), protein trafficking, and lipid biosynthesis. In addition, IRE1 RNase activity is involved in the degradation of other RNAs (including mRNAs and microRNAs) in a process termed regulated IRE1-dependent decay (RIDD) that promotes both pro-survival or pro-death features [8]. PERK is a serine/threonine kinase that is activated through oligomerization and *trans*-autophosphorylation upon release of GRP78. Activated PERK phosphorylates its main substrate, the eukaryotic translation initiation factor 2α (eIF2α), on Ser51. This results in global protein synthesis attenuation and in the selective translation of ATF4, a transcription factor that regulates specific metabolic programs such as glucose homeostasis and amino acid synthesis. ATF6 is a membrane-anchored transcription factor. Upon ER stress, the coordinated dissociation of GRP78 induces ATF6 export to the Golgi complex where it is cleaved by site-1 and site-2 proteases (S1P and S2P). The released cytosolic domain is a transcription factor that translocates to the nucleus where it triggers the expression of genes encoding chaperones, components of the ERAD machinery, and proteins involved in lipid metabolism [1,12]. Abbreviations: CRT, calreticulin; DAMP, danger-associated molecular pattern; HMGB1, high mobility group box 1; ICD, immunogenic cell death; P, phosphorylation; sXBP1 mRNA, spliced *XBP1* mRNA; TERS, transmissible ER stress; uXBP1 mRNA, unspliced *XBP1* mRNA.

Box 2. The UPR and Cytokine Regulation

In response to ER stress, CCAAT-enhancer-binding protein homologous protein (CHOP) acts as a transcription factors to control the production of interleukin (IL)-23 in DCs, and sXBP1 induces tumor necrosis factor (TNF-α) in macrophages [40,41]. IRE1 is implicated in inflammation by activating c-Jun N-terminal kinase (JNK), inducing IL-8 production under glutamine deprivation conditions [42] and IL-1β through the activation of glycogen synthase kinase 3β (GSK3β) [43]. Cytokines can also trigger ER stress. For example, IκB kinase (IKKβ) phosphorylates sXBP1 in response to TNF-α, increasing its activity [44]. In addition, IL-10-mediated p38 signaling can block ER stress by inhibiting the TNF-dependent translocation of the cleaved fragment of activating transcription factor 6 (ATF6) into the nucleus and its recruitment to the 78 kDa glucose-regulated protein (*GRP78*) promoter [45]. IL-1, −6, −8, and TNF activate the UPR and initiate a systemic acute inflammatory response by modulating acute-phase response genes in mouse hepatocytes in the liver *in vivo* through increased cleavage of the ER-localized transcription factor, cAMP response element-binding protein (CREBH) [46]. Moreover, type 2 T helper cell (Th2)-associated cytokines (IL-4, IL-6, and IL-10) activate the IRE1 pathway through STAT3 and STAT6, thus upregulating cathepsin secretion by macrophages. Pharmacological inhibition of IRE1 blocks cathepsin secretion and macrophage-mediated cancer cell invasion [47]. Altogether, the feedback between ER stress, UPR induction, and cytokine production results in enhanced inflammatory responses.

Box 3. Transmissible ER Stress

Another way by which ER stress can modulate the immune system is known as TERS. Upon UPR induction cells release TERS molecules that can transmit ER stress to other recipient cells either through proteinaceous signals or membrane-containing structures (extracellular vesicles). TERS is a new phenomenon and its nature remains to be fully characterized. When human and murine prostate cancer cell lines were cultured in the presence of conditioned media generated from cells treated with the ER stress- and UPR-inducer thapsigargin, their survival was increased relative to cells cultured in control media in the face of stress insults such as chemotherapy and glucose deprivation through the activation of the PERK branch of the UPR [48]. This is thought to be linked to the production of TERS molecules by cancer cells treated with UPR inducers; however, the exact nature of such structures remains to be clarified. TERS has also been observed between lung tumor murine cancer cells and immune system cells. TLR4-dependent TERS was described in macrophages cultured in conditioned media of cancer cells treated with staurosporine. These macrophages were driven towards a proinflammatory phenotype, facilitating cancer progression [49,50]. Tumor ER stress has also been shown to be transmitted to bone marrow-derived dendritic cells (BMDCs). Conditioned media of prostate, melanoma, and Lewis lung carcinoma cells treated with thapsigargin induced ER stress and the UPR in BMDCs, leading to the production of proinflammatory cytokines by these cells. In these settings there was impaired antigen cross-presentation by TERS-imprinted BMDCs. $CD8^+$ T cells cross-primed by TERS-imprinted BMDCs showed very high splicing of *XBP1* compared to other UPR elements and very little proliferation capacity. The role of the activation of the IRE1–XBP1 axis on the cell function of the $CD8^+$ T cells remains to be elucidated [50]. However, most studies aiming to analyze TERS were carried out in response to pharmacological ER stressors in cancer cells, and these studies suggest a protumoral role for TERS. Conversely, some types of chemotherapy that induce ER stress in cancer cells have been described to have antitumoral immune effects [30]. The question remains as to whether non-pharmacological/more physiologically relevant stimuli such as those occurring in the TME induce the same type of TERS? Do they have the same effect on cancer progression, or is there an antitumoral type of TERS? Further studies to fill this gap are needed.

Altogether, understanding the regulation of ER stress and the UPR in the context of cancer is vital to find new ways to restore cell immunogenicity and potentiate the immune system to improve the response to therapies.

Nutritional Stress, IRE1 Signaling, and the Immune Response: Deeper into the Rabbit Hole

It has been shown that a decrease in calorie or food intake can induce **autophagy**-dependent anticancer immunosurveillance [32]. Unfortunately, the use of dietary restriction (DR) to reduce tumor growth can be very hard on cancer patients owing to the risk of cachexia and DR-related weight loss. For this reason, effort is being put into the development of different ways to mimic the benefits of DR on tumor growth, but without impacting on calorie or food intake. Previous studies have shown that changes in amino acid availability can induce ER stress. For example, proline deprivation induces ER stress and the IRE1 arm of the UPR, impairing the clonogenic and tumorigenic potential of a vast panel of breast, cervix, esophagus, lung, skin, ovary, pancreas, and stomach proline-dependent tumor cells both *in vitro* and in immunodeficient mice [33]. These results indicate that the effect of a strong reduction in amino acid availability is

not related to the immune response but directly affects the ability of tumor cells to proliferate. Mice bearing proline-dependent tumors showed decreased glutamine and ornithine plasma concentrations compared to mice bearing proline-independent tumors, thus suggesting increased conversion of glutamine or ornithine into proline to compensate for the lack of dietary proline [33]. Alternatively, macronutrient modulation through a mild reduction in protein intake reduced the relative levels of all amino acids, except for glutamine, and induced the IRE1 pathway in tumor cells in a xenograft model of colorectal cancer. This IRE1-specific activation of the UPR in tumor cells triggered the immune system and led to increased immunosurveillance [25]. Nevertheless, the exact mechanism linking a low-protein diet to IRE1 activation remains to be elucidated. One can speculate that the reduction in specific amino acids may slow down protein folding in highly dividing cells, therefore inducing IRE1 activation. In line with this, it has been shown in *E. coli* and in human cells that silent mutations in EgFABP1 (*Echinococcus granulosus* fatty acid-binding protein 1) [34] and in *MDR1* (multidrug resistance 1) [35], respectively, resulted in changed translation rates, therefore altering protein folding leading to the UPR. Interestingly, the percentage of protein reduction in the diet determines if its effect is increased immunosurveillance or immune system evasion. Notably, the protective effect of the low-protein diet (Low PROT 12.5% – CHO 72.2%, PROT 17%, FAT 10.8%; Low PROT 25% – CHO 73.7%, PROT 14.9%, FAT 11.5%) compared to the control diet (control – CHO 70.9%, PROT 19.5%, FAT 9.6%) was lost when mice were fed with a very low protein diet (Low PROT 40% – CHO 76.4%, PROT 12.2%, FAT 11.4%) [25]. We hypothesize that such a massive reduction in protein may prevent the induction of the anticancer immune response by activating additional signaling pathways that remain to be identified.

These results are appealing because they suggest that there is a threshold for the decrease in dietary protein that determines the nature of the immune response. They also reflect the need for consensus on what to consider as a low-protein diet, which is one of the reasons why obtaining human data from trials testing low protein intake can be difficult. An additional reason why translating these studies into the clinic is highly speculative lies in the fact that rodent metabolism is very different from that of humans. Only a careful and multicentric clinical investigation will determine the potential beneficial effect of such a low-protein diet on patients. Conversely, other studies have shown a detrimental effect of the activation of IRE1 on mouse survival, confirming the pro-survival effect of the pathway on cancer cells. Pharmacological inhibition of the IRE1/XBP1 pathway suppresses the growth of patient-derived MYC-over-expressing breast tumors in immunodeficient SCID beige mice compared to vehicle treatment [36]. Similarly, XBP1 has been shown to negatively regulate ICD in colorectal CT26 tumors expressing human EGFR growing in immunocompetent BALB/c mice [37]. ICD induction depended on the mutational status of the EGFR signaling pathway and was restored upon the inhibition of XBP1. Similarly, the IRE1/XBP1 pathway is induced in MYC-overexpressing Burkitt lymphoma, neuroblastoma, and hepatocellular carcinoma cells, leading to sustained growth and survival *in vitro* and in immunocompetent mice, and pharmacological and genetic inhibition of sXBP1 induced MYC-dependent apoptosis [38].

A low-protein diet, which induces IRE1 signaling, also had a detrimental effect on Eμ–Myc lymphoma progression in immunodeficient NSG mice. It led to a decrease in mouse survival compared to mice fed with a control diet, confirming the pro-survival role of IRE1 in MYC-positive tumor cells in the absence of the immune system. Conversely, when the same experiments on Eμ–Myc lymphoma progression were performed in immunocompetent C57BL/6 mice, the immune system changed the outcome because the low-protein diet-dependent induction of IRE1 resulted in an anticancer immune response [25]. Altogether, not only the cell type nor the stimulus inducing the pathway (e.g., ER stress, nutritional stress,

Box 4. Clinician's Corner

Cancer aggressiveness and response to therapy depend on its capacity to activate appropriate adaptive response pathways. Modulation of the UPR enables tumor cells to respond to environmentally harsh conditions by escaping or inducing cell death, and allows crosstalk with the immune system.

The intensity and features of the UPR vary among tissues, and an upregulated UPR can correlate with the severity of cancer. However, the functions and effects of the UPR cannot be directly translated from one cell type to another because the outcome may be cell type-dependent.

The UPR is crucial for ICD regulation and is important for long-term effectiveness of anticancer treatment.

In recent years many compounds interfering with the UPR have been studied, with very promising results. Owing to growing knowledge on the complexity of the UPR landscape among cancer stages and types, further studies will be necessary to unveil the potential of therapies based on UPR modulation and to stratify the patient populations to be treated.

oncogenic mutations, etc.) but also the integrity and status of the immune system can determine and explain the differences observed in these studies regarding the effect of IRE1 induction on tumor progression. Supporting the hypothesis of its dual role, pharmacological and genetic inhibition of the IRE1/RIDD/RIG-I pathway reverted the effect of a low-protein diet, resulting in sustained tumor growth [25]. This shows the potential power of tumor/immune system crosstalk in determining disease outcome. These studies highlight the capacity of the IRE1 branch of the UPR to control tumor cell crosstalk with the immune system, and indicate that understanding the underlying mechanisms may open new potential ways of treating cancer.

Concluding Remarks

The TME plays an important role in tumor development because it conditions drug resistance and cell survival. Chemotherapy, poor vascularization, oxygen and nutrient availability can induce stress pathways, including the UPR, that counteract these stressors at the same time impacting on TME. The UPR regulates the crosstalk between tumor cells and the immune system, potentially opening a new area of research (see Outstanding Questions and Box 4). IRE1 can either play pro- or antitumoral roles but what determines which role it takes still remains an open question. It could depend on the stimulus (e.g., oncogene driven protein synthesis, reactive oxygen species, hypoxia, nutrient shortage, chemotherapy, among others), on the complex balance of expression of the proteins induced downstream of the UPR sensors and/or on the intrinsic nature of the cells within the context of their environment. Understanding specific IRE1 activation and ER stress dynamics in the TME could offer new possibilities for improved and effective anticancer therapies.

Outstanding Questions

We have focused on the dual effects of UPR activation on tumor progression. The different UPR pathways can be both pro- and antitumorigenic depending on the cell type, the stimulus, and its duration. How is this machinery fine-tuned, and what determines the final outcome of UPR induction?

Because short-term UPR induction can be pro-survival, whereas chronic UPR activation can lead to cell death, how can we make wise use of UPR-modulating compounds so as to achieve the greatest benefits for cancer treatment?

Which pathways of the UPR are responsible for immune system activation, and which are responsible for immune system suppression? The UPR is not exclusively activated by misfolded protein accumulation. Other factors such as membrane lipid dysregulation, cytokines, and micronutrient and macronutrient deprivation can also induce the UPR. Does the source of UPR activation affect the nature of the immune response?

The ER controls the majority of secreted proteins and proteins exposed on the cell membrane, therefore regulating tumor immunogenicity. Is this the primary mechanism by which the UPR controls the immune response? How big is the impact of TERS on the immune response?

Acknowledgments

Our work is supported by grants from the Fondation ARC pour la Recherche sur le Cancer (ARC), the Agence Nationale de la Recherche (ANR; LABEX SIGNALIFE ANR-11-LABX-0028-01), and research funding from the Cancéropôle Provence-Alpes–Côte d'Azur (PACA) to J.E.R., and from the Institut National du Cancer (INCA; PLBIO2017), EU H2020 Marie Skłodowska-Curie Action (MSCA) ITN-675448 (TRAINERS), and MSCARISE-734749 (INSPIRED) to E.C.

References

1. Hetz, C. *et al.* (2015) Proteostasis control by the unfolded protein response. *Nat. Cell Biol.* 17, 829–838
2. Cubillos-Ruiz, J.R. *et al.* (2017) Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. *Cell* 168, 692–706
3. Higuchi-Sanabria, R. *et al.* (2018) A futile battle? Protein quality control and the stress of aging. *Dev. Cell* 44, 139–163
4. Hart, L.S. *et al.* (2012) ER stress-mediated autophagy promotes Myc-dependent transformation and tumor growth. *J. Clin. Invest.* 122, 4621–4634

5. Ma, Y. and Hendershot, L.M. (2004) The role of the unfolded protein response in tumour development: friend or foe? *Nat. Rev. Cancer* 4, 966–977
6. Obacz, J. *et al.* (2017) Endoplasmic reticulum proteostasis in glioblastoma – from molecular mechanisms to therapeutic perspectives. *Sci. Signal.* 10
7. Denoyelle, C. *et al.* (2006) Anti-oncogenic role of the endoplasmic reticulum differentially activated by mutations in the MAPK pathway. *Nat. Cell Biol.* 8, 1053–1063
8. Huber, A.L. *et al.* (2013) p58(IPK)-mediated attenuation of the proapoptotic PERK–CHOP pathway allows malignant progression upon low glucose. *Mol. Cell* 49, 1049–1959
9. Han, D. *et al.* (2009) IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. *Cell* 138, 562–575
10. Maurel, M. *et al.* (2014) Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem. Sci.* 39, 245–254
11. Tavernier, S.J. *et al.* (2017) Regulated IRE1-dependent mRNA decay sets the threshold for dendritic cell survival. *Nat. Cell Biol.* 19, 698–710
12. Wang, M. and Kaufman, R.J. (2014) The impact of the endoplasmic reticulum protein-folding environment on cancer development. *Nat. Rev. Cancer* 14, 581–597
13. Iurlaro, R. and Munoz-Pinedo, C. (2016) Cell death induced by endoplasmic reticulum stress. *FEBS J.* 283, 2640–2652
14. Li, X.X. *et al.* (2017) Knockdown of IRE1alpha inhibits colonic tumorigenesis through decreasing beta-catenin and IRE1alpha targeting suppresses colon cancer cells. *Oncogene* 36, 6738–6746
15. Cubillos-Ruiz, J.R. *et al.* (2015) ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. *Cell* 161, 1527–1538
16. Osorio, F. *et al.* (2014) The unfolded-protein-response sensor IRE-1alpha regulates the function of CD8alpha$^+$ dendritic cells. *Nat. Immunol.* 15, 248–257
17. Condamine, T. *et al.* (2016) Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci. Immunol.* 1, aaf8943
18. Grootjans, J. *et al.* (2016) The unfolded protein response in immunity and inflammation. *Nat. Rev. Immunol.* 16, 469–484
19. Shi, W. *et al.* (2015) Transcriptional profiling of mouse B cell terminal differentiation defines a signature for antibody-secreting plasma cells. *Nat. Immunol.* 16, 663–673
20. Lu, Z. *et al.* (2017) Fasting selectively blocks development of acute lymphoblastic leukemia via leptin-receptor upregulation. *Nat. Med.* 23, 79–90
21. Bujisic, B. *et al.* (2017) Impairment of both IRE1 expression and XBP1 activation is a hallmark of GCB DLBCL and contributes to tumor growth. *Blood* 129, 2420–2428
22. Carrasco, D.R. *et al.* (2007) The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. *Cancer Cell* 11, 349–360
23. Lhomond, S. *et al.* (2018) Dual IRE1 RNase functions dictate glioblastoma development. *EMBO Mol. Med.* 10, e7929
24. Eckard, S.C. *et al.* (2014) The SKIV2L RNA exosome limits activation of the RIG-I-like receptors. *Nat. Immunol.* 15, 839–845
25. Rubio-Patino, C. *et al.* (2018) Low-protein diet induces IRE1alpha-dependent anticancer immunosurveillance. *Cell Metab.* 27, 828–842
26. Hou, J. *et al.* (2014) Hepatic RIG-I predicts survival and interferon-alpha therapeutic response in hepatocellular carcinoma. *Cancer Cell* 25, 49–63
27. Dassler-Plenker, J. *et al.* (2016) RIG-I activation induces the release of extracellular vesicles with antitumor activity. *Oncoimmunology* 5, e1219827
28. Duewell, P. *et al.* (2014) RIG-I-like helicases induce immunogenic cell death of pancreatic cancer cells and sensitize tumors toward killing by CD8$^+$ T cells. *Cell Death Differ.* 21, 1825–1837
29. Dejeans, N. *et al.* (2014) Addicted to secrete – novel concepts and targets in cancer therapy. *Trends Mol. Med.* 20, 242–250
30. Galluzzi, L. *et al.* (2017) Immunogenic cell death in cancer and infectious disease. *Nat. Rev. Immunol.* 17, 97–111
31. Obacz, J. *et al.* (2017) Regulation of tumor-stroma interactions by the unfolded protein response. *FEBS J.* 14,Published online December 14, 2017. http://dx.doi.org/10.1111/febs.14359
32. Pietrocola, F. *et al.* (2016) Caloric restriction mimetics enhance anticancer immunosurveillance. *Cancer Cell* 30, 147–160
33. Sahu, N. *et al.* (2016) Proline starvation induces unresolved ER stress and hinders mTORC1-dependent tumorigenesis. *Cell Metab.* 24, 753–761
34. Cortazzo, P. *et al.* (2002) Silent mutations affect in vivo protein folding in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* 293, 537–541
35. Kimchi-Sarfaty, C. *et al.* (2007) A 'silent' polymorphism in the MDR1 gene changes substrate specificity. *Science* 315, 525–528
36. Zhao, N. *et al.* (2018) Pharmacological targeting of MYC-regulated IRE1/XBP1 pathway suppresses MYC-driven breast cancer. *J. Clin. Invest.* 128, 1283–1299
37. Pozzi, C. *et al.* (2016) The EGFR-specific antibody cetuximab combined with chemotherapy triggers immunogenic cell death. *Nat. Med.* 22, 624–631
38. Xie, H. *et al.* (2018) IRE1alpha RNase-dependent lipid homeostasis promotes survival in Myc-transformed cancers. *J. Clin. Invest.* 128, 1300–1316
39. Dejeans, N. *et al.* (2015) Novel roles of the unfolded protein response in the control of tumor development and aggressiveness. *Semin. Cancer Biol.* 33, 67–73
40. Goodall, J.C. *et al.* (2010) Endoplasmic reticulum stress-induced transcription factor, CHOP, is crucial for dendritic cell IL-23 expression. *Proc. Natl. Acad. Sci. U. S. A.* 107, 17698–17703
41. Martinon, F. *et al.* (2010) TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. *Nat. Immunol.* 11, 411–418
42. Shanware, N.P. *et al.* (2014) Glutamine deprivation stimulates mTOR–JNK-dependent chemokine secretion. *Nat. Commun.* 5, 4900
43. Kim, S. *et al.* (2015) Endoplasmic reticulum stress-induced IRE1-alpha activation mediates cross-talk of GSK-3beta and XBP-1 to regulate inflammatory cytokine production. *J. Immunol.* 194, 4498–4506
44. Liu, J. *et al.* (2016) Inflammation improves glucose homeostasis through IKKbeta-XBP1s interaction. *Cell* 167, 1052–1066
45. Shkoda, A. *et al.* (2007) Interleukin-10 blocked endoplasmic reticulum stress in intestinal epithelial cells: impact on chronic inflammation. *Gastroenterology* 132, 190–207
46. Zhang, K. *et al.* (2006) Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* 124, 587–599
47. Yan, D. *et al.* (2016) STAT3 and STAT6 signaling pathways synergize to promote cathepsin secretion from macrophages via IRE1alpha activation. *Cell Rep.* 16, 2914–2927
48. Rodvold, J.J. *et al.* (2017) Intercellular transmission of the unfolded protein response promotes survival and drug resistance in cancer cells. *Sci. Signal.* 10, eaah7177
49. Mahadevan, N.R. *et al.* (2011) Transmission of endoplasmic reticulum stress and pro-inflammation from tumor cells to myeloid cells. *Proc. Natl. Acad. Sci. U. S. A.* 108, 6561–6566
50. Mahadevan, N.R. *et al.* (2012) Cell-extrinsic effects of tumor ER stress imprint myeloid dendritic cells and impair CD8$^+$ T cell priming. *PLoS One* 7, e51845

4. Article 4:

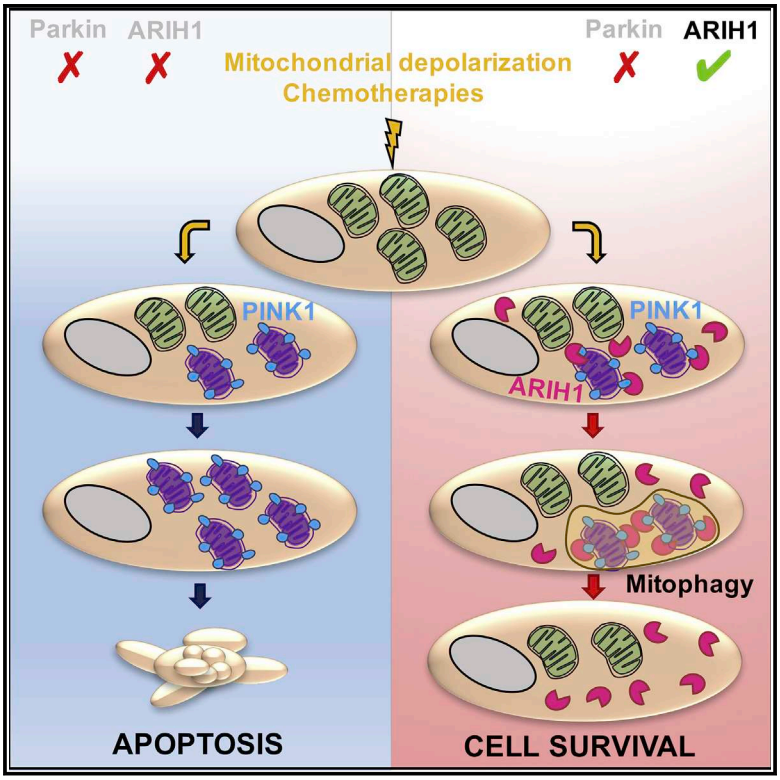
Villa, E., E. Proics, C. Rubio-Patino, S. Obba, B. Zunino, **J. P. Bossowski**, R. M. Rozier, J. Chiche, L. Mondragon, J. S. Riley, S. Marchetti, E. Verhoeyen, S. W. G. Tait and J. E. Ricci (2017). "Parkin-Independent Mitophagy Controls Chemotherapeutic Response in Cancer Cells." Cell Rep 20(12): 2846-2859.

Article

Cell Reports

Parkin-Independent Mitophagy Controls Chemotherapeutic Response in Cancer Cells

Graphical Abstract



Highlights

- Parkin (RBR E3 ligase) is absent in most cancer cells when mitophagy is functional
- ARIH1, an E3 ligase belonging to the RBR family, is expressed in cancer cells
- ARIH1 controls mitophagy of damaged mitochondrial in a PINK1-dependent manner
- ARIH1's control of mitophagy protects cancer cells from chemotherapy-induced death

Authors

Elodie Villa, Emma Proïcs, Camila Rubio-Patiño, ..., Els Verhoeyen, Stephen W.G. Tait, Jean-Ehrland Ricci

Correspondence

ricci@unice.fr

In Brief

Clearance of damaged mitochondria (mitophagy) is involved in the resistance to chemotherapeutic-induced death, but the main known regulators of mitophagy are not expressed in cancer cells. Villa et al. show that the RBR E3 ligase ARIH1 is expressed in several cancer cell types. ARIH1 controls PINK1-dependent mitophagy and sensitivity to chemotherapies.

Villa et al., 2017, Cell Reports *20*, 2846–2859
September 19, 2017
http://dx.doi.org/10.1016/j.celrep.2017.08.087

OPEN ACCESS
CellPress

Cell Reports
Article

Parkin-Independent Mitophagy Controls Chemotherapeutic Response in Cancer Cells

Elodie Villa,[1] Emma Proïcs,[1] Camila Rubio-Patiño,[1] Sandrine Obba,[1] Barbara Zunino,[1] Jozef P. Bossowski,[1] Romain M. Rozier,[1] Johanna Chiche,[1] Laura Mondragón,[1] Joel S. Riley,[2] Sandrine Marchetti,[1] Els Verhoeyen,[1] Stephen W.G. Tait,[2] and Jean-Ehrland Ricci[1,3,*]

[1]Université Côte d'Azur, INSERM, C3M, Nice, France
[2]Cancer Research UK Beatson Institute, Institute of Cancer Sciences, University of Glasgow, Switchback Road, Glasgow, UK
[3]Lead Contact
*Correspondence: ricci@unice.fr
http://dx.doi.org/10.1016/j.celrep.2017.08.087

SUMMARY

Mitophagy is an evolutionarily conserved process that selectively targets impaired mitochondria for degradation. Defects in mitophagy are often associated with diverse pathologies, including cancer. Because the main known regulators of mitophagy are frequently inactivated in cancer cells, the mechanisms that regulate mitophagy in cancer cells are not fully understood. Here, we identified an E3 ubiquitin ligase (ARIH1/HHARI) that triggers mitophagy in cancer cells in a PINK1-dependent manner. We found that ARIH1/HHARI polyubiquitinates damaged mitochondria, leading to their removal via autophagy. Importantly, ARIH1 is widely expressed in cancer cells, notably in breast and lung adenocarcinomas; ARIH1 expression protects against chemotherapy-induced death. These data challenge the view that the main regulators of mitophagy are tumor suppressors, arguing instead that ARIH1-mediated mitophagy promotes therapeutic resistance.

INTRODUCTION

Mitochondria are essential for energy production, reactive oxygen species (ROS) production, calcium buffering, and regulation of several forms of cell death (Villa and Ricci, 2016; Wallace, 2005). Over time, or in response to various stresses, mitochondria will accumulate damage. Therefore, cells have adopted several quality-control processes, including cycles of mitochondrial fusion and fission and the selective elimination of dysfunctional mitochondria by mitophagy, an organelle-specific type of macroautophagy, to maintain a functional network of healthy mitochondria (Wei et al., 2015).

The ubiquitin (Ub) E3 ligase Parkin, which is mutated in recessive familial forms of Parkinson's disease, is a key mediator of mitochondrial quality control processes (Kitada et al., 1998; Shimura et al., 2000). Phosphatase and tensin homolog deleted on chromosome 10-induced kinase 1 (PINK1) is a serine/threonine kinase that shuttles between the cytosol and mitochondria in healthy cells. Normally, it is rapidly degraded by mitochondrial proteases, but PINK1 can stabilize on the outer membrane of depolarized mitochondria and recruit Parkin, which is initially inactive (Clark et al., 2006; Narendra et al., 2008; Park et al., 2006). PINK1 will phosphorylate Parkin on the Ub-like (UBL) domain on the Ser^{65} in a $\Delta\Psi$m-dependent process, resulting in an increase of its Ub ligase activity and the formation of polyubiquitin chains on the surface of depolarized mitochondrial membranes. PINK1 will also phosphorylate the conserved Ser^{65} site of Ub molecule (Kane et al., 2014; Kazlauskaite et al., 2015; Koyano et al., 2014; Wauer et al., 2015). It has been proposed that the phosphorylated Ub could act as a Parkin activator by overcoming the autoinhibitory mechanism of Parkin. Both events are needed to fully activate Parkin, which will, in turn, polyubiquitinate numerous mitochondrial outer membrane proteins, leading to the recruitment of the Ub- and LC3-binding adaptor p62 to these damaged organelles (Herhaus and Dikic, 2015). While p62 was initially shown to be critical for removing depolarized mitochondria by transporting them to autophagosomes, later studies suggested a possible redundancy with the related Ub- and Atg8/LC3II-binding protein NBR1 (Narendra et al., 2010; Okatsu et al., 2010), NDP52 (nuclear domain 10 protein 52, also known as CALCOCO2), or optineurin (Lazarou et al., 2015). In particular, NDP52 and optineurin recognize phospho-Ub, leading to the recruitment of the autophagy machinery to initiate mitophagy (Lazarou et al., 2015).

Loss of either PINK1 or Parkin leads to accumulation of damaged mitochondria in several models (fly, mouse, and human), further supporting their central and conserved role in mitochondrial quality-control pathways (Herhaus and Dikic, 2015).

A growing body of evidence has shown the involvement of somatic Parkin inactivation in a broad panel of human cancers. Indeed, Parkin has been shown to be downregulated in multiple cancer cell lines and primary tumors (Gong et al., 2014; Veeriah et al., 2010a, 2010b). Parkin-deficient mice show increased susceptibility to tumorigenesis, while ectopic Parkin expression reduces the in vitro or in vivo growth of cancer cells of various origins, strongly suggesting a tumor-suppressive role for Parkin (for review, see Xu et al., 2014). Therefore, as Parkin is often downregulated in tumors, the molecular events that promote mitophagy in these cells remain to be determined.

Ub and Ub-like modifications occur in a three-step enzymatic process. E1 is an activating enzyme that forms a thioester bond with the Ub protein. Then, the charged Ub monomer is transferred to an E2 enzyme that conjugates the Ub molecule to its target protein, with the help of an E3 Ub ligase (Kerscher

et al., 2006; Nagy and Dikic, 2010). While there are few E1 and E2 ligases, there are many diverse E3 Ub ligases that control substrate specificity and are responsible for the enormous diversity of the Ub system. Several different classes of E3 Ub ligases have been identified. The RING ubiquitinases function as a scaffold between the E2 ligase and the substrate, allowing the transfer of the Ub moiety to the target protein. In contrast, homologous to the E6-AP carboxyl terminus (HECT) ubiquitinases play a direct role in substrate ubiquitination by forming a catalytic intermediate thioester between the C-lobe cysteine residue and the C terminus of Ub (Spratt et al., 2014). Recently, E3 ligases from the Parkin family were classified as hybrids between RING and HECT and were therefore referred to as RING-between-RING (RBR) E3 ligases (Wenzel et al., 2011). They are composed of a canonical RING domain, an in-between ring fingers (IBR) domain, and a RING2 domain. This family has 14 members, including Parkin and Ariadne RBR E3 Ub protein ligase 1 (ARIH1), also known as HHARI. For these ligases, the first RING domain of the RBR module does not directly transfer an E2-bound Ub onto a substrate but instead transfers it to a Cys residue in the RING2 domain (Kulathu and Komander, 2012).

Here, we explored the mechanism controlling mitophagy in cancer cells. As Parkin is not expressed in most cancer cells, we investigated how mitophagy could occur in these cells.

RESULTS

ARIH1 Expression Promotes Elimination of Depolarized Mitochondria

Parkin is a member of the RBR family of E3 ligases that is composed of 14 complex multidomain enzymes. As it is frequently downregulated in cancer cells, we investigated whether other E3 ligases could control mitophagy in these cells. We hypothesized that another member of the RBR family could possibly fulfill this function. A survey of the different family members led us to focus on ARIH1, as it shares the same E2 ligase as Parkin (UbcH7, also known as UBE2L3) (Wenzel et al., 2011), and because an elegant study using a small interfering RNA (siRNA)-based screen recently determined that this E3 ligase is involved in the protection of cancer cells against genotoxic stress (von Stechow et al., 2015).

Depolarization of the mitochondria in HeLa cells using the protonophore carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) promoted mitophagy of damaged mitochondria only upon expression of Parkin (Figure 1A). Importantly, removal of damaged mitochondria was also observed following ARIH1 overexpression (Figure 1A). Indeed, after 6 hr of CCCP treatment, we observed mitochondrial network collapse around the perinuclear region in HeLa cells expressing ARIH1, as determined by TOM20 staining (Figure S1A), while after 24 hr of CCCP treatment, we observed a complete loss of the mitochondrial marker TOM20 (Figures 1A–1C) and a strong reduction in COX IV, succinate dehydrogenase iron-sulfur subunit (SDHB), and NDUFB8 expression, mitochondrial proteins that are typically degraded during mitophagy (Figures 1D and 1E). We observed that ARIH1-dependent mitophagy occurred to the same extent as Parkin-dependent mitophagy (Figures 1D and 1E). Importantly, as described for Parkin, ARIH1 was recruited to mitochondria upon CCCP treatment (Figure S1A). Thus, we concluded that ARIH1 overexpression led to the removal of depolarized mitochondria.

ARIH1-Mediated Removal of Damaged Mitochondria Occurs via Mitophagy

We addressed whether the ARIH1-mediated removal of damaged mitochondria involved mitophagy. To establish this point, we measured mitophagy using m-Keima fluorophore, a biosensor of mitochondrial degradation by the lysosomes (Katayama et al., 2011). m-Keima is a variant of RFP that is targeted to the mitochondrial matrix. This cellular biomarker changes its fluorescence profile in response to pH and is resistant to degradation within lysosomes. As presented in Figures 2A and 2B, we measured m-Keima conversion from green (488 nm) to red (561 nm) fluorescence during treatment with several mitochondria-damaging agents, such as CCCP, oligomycin/antimycin (O/A), and valinomycin, using fluorescence-activated cell sorting (FACS) (Lazarou et al., 2015). We observed that ARIH1 could mediate mitophagy upon mitochondrial damage to the same extent as Parkin overexpression. ARIH1-mediated mitophagy upon mitochondrial damage was confirmed by the strong reduction of several mitochondrial markers, such as SDHB and NDUFB8, at the protein level (Figure 2C). Interestingly, we observed that basal mitophagy that removes damaged mitochondria produced over time was also dependent on ARIH1 expression (Figures S1B and S1C).

To further characterize ARIH1-dependent mitophagy induction, we measured autophagic flux in ARIH1- and Parkin-overexpressing HeLa cells following CCCP treatment. We observed an increase of the autophagic flux as determined by the increased conversion of LC3B-I to LC3B-II and increased degradation of the autophagy receptor SQSTM1/p62 and the mitochondrial protein COX IV (Figures S2B and S2C) in the presence of ARIH1 or Parkin overexpression. Importantly, treatment with the lysosomal inhibitor bafilomycin A1 (BafA1) further increased LC3B-II accumulation and reduced p62 and COX IV degradation, indicating a complete autophagic response (Figures S2B and S2C). We verified that CCCP had no effect on the expression of those proteins in the absence of ARIH1 or Parkin expression (Figure S2A). We also observed that LC3, ARIH1 (or Parkin used as a positive control), and the mitochondrial marker cytochrome *c* were co-localized on mitochondria upon CCCP treatment (Figures S2D and S2E), suggesting that ARIH1 mediates mitophagy upon mitochondrial damage. To strengthen this point, we observed that in HeLa cells transfected with an empty vector, LC3 was not co-localized on mitochondria upon CCCP treatment (Figure S2F), confirming that HeLa cells cannot perform mitophagy to a significant extent in the absence of one of those E3 ligases.

To further investigate the role macroautophagy in ARIH1-dependent mitophagy, we used ATG7 knockout (KO) mouse embryonic fibroblasts (MEFs) and KO MEFs reconstituted with ATG7-GFP (R-ATG7 MEFs; Figures 2D, 2E, and S3) (Taherbhoy et al., 2011). Importantly, MEFs (KO and reconstituted) expressed equivalent endogenous levels of ARIH1 but did not

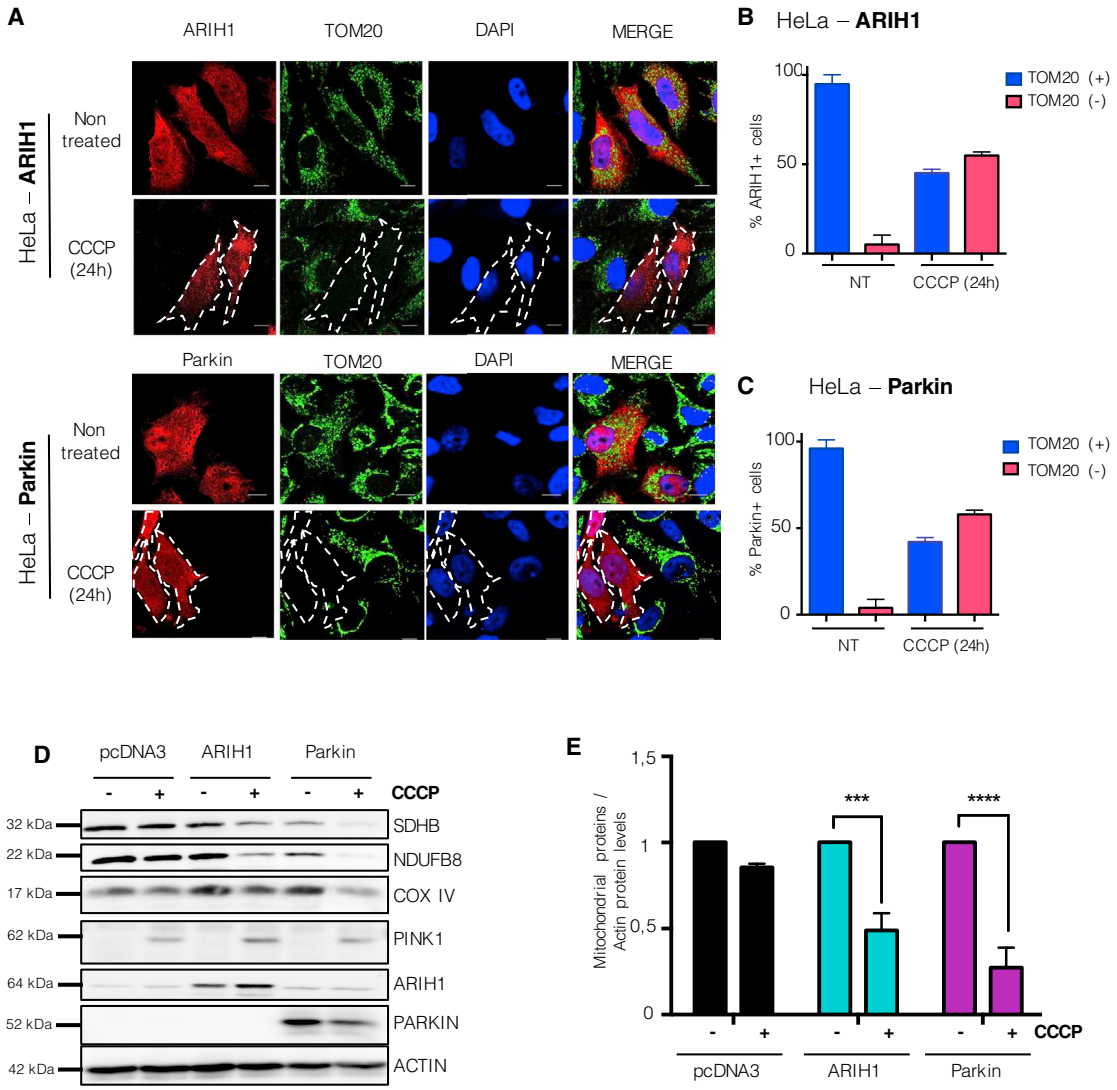


Figure 1. ARIH1 Promotes the Removal of Damaged Mitochondria

HeLa cells were transfected to transiently overexpress the control vector (pcDNA$_3$), ARIH1, or Parkin and then treated with the mitochondrial uncoupling agent CCCP (10 μM) for 24 hr.

(A–C) Mitochondria were immunostained for TOM20 (green), and the absence of the mitochondrial marker TOM20 was assessed in ARIH1$^+$ cells (B) or in Parkin$^+$ cells (C) using confocal microscopy (scale bar, 10 μm). Quantification of mitophagy was estimated by counting a minimum of 100 cells for each condition. Data are shown as the mean of 3 independent experiments.

(D) Whole-cell lysates were analyzed for indicated protein expression by immunoblotting (actin was used as a loading control).

(E) Data are shown as the mean ± SEM of 3 independent experiments.

***$p < 0.001$ and ****$p < 0.0001$ according to a two-way ANOVA.

express Parkin (Figures 2D and S3). We observed that endogenous expression of ARIH1 in MEFs was sufficient to decrease SDHB expression (Figures 2D and 2E) following CCCP treatment in reconstituted MEFs. However, this effect was not observed in ATG7 KO MEFs. We also verified that knockdown of endogenous ARIH1 expression using siRNA prevented ARIH1-dependent mitophagy upon CCCP treatment in R-ATG7 MEFs.

Collectively, these data demonstrate that ARIH1 mediates the removal of depolarized mitochondria through mitophagy.

ARIH1-Mediated Mitophagy Is Dependent on Its Ub Ligase Activity and PINK1 Stabilization

To further investigate the molecular mechanisms underlying ARIH1-mediated autophagy, we evaluated the contribution of PINK1. To accomplish this, we first knocked down PINK1 expression using siRNAs in ARIH1-overexpressing HeLa cells. As shown in Figures 3A and 3B, silencing of PINK1 prevented the COX IV decrease following CCCP treatment in HeLa cells overexpressing ARIH1. The involvement of PINK1 was further tested by knocking out PINK1 using CRISPR/Cas9 (Figures 3C

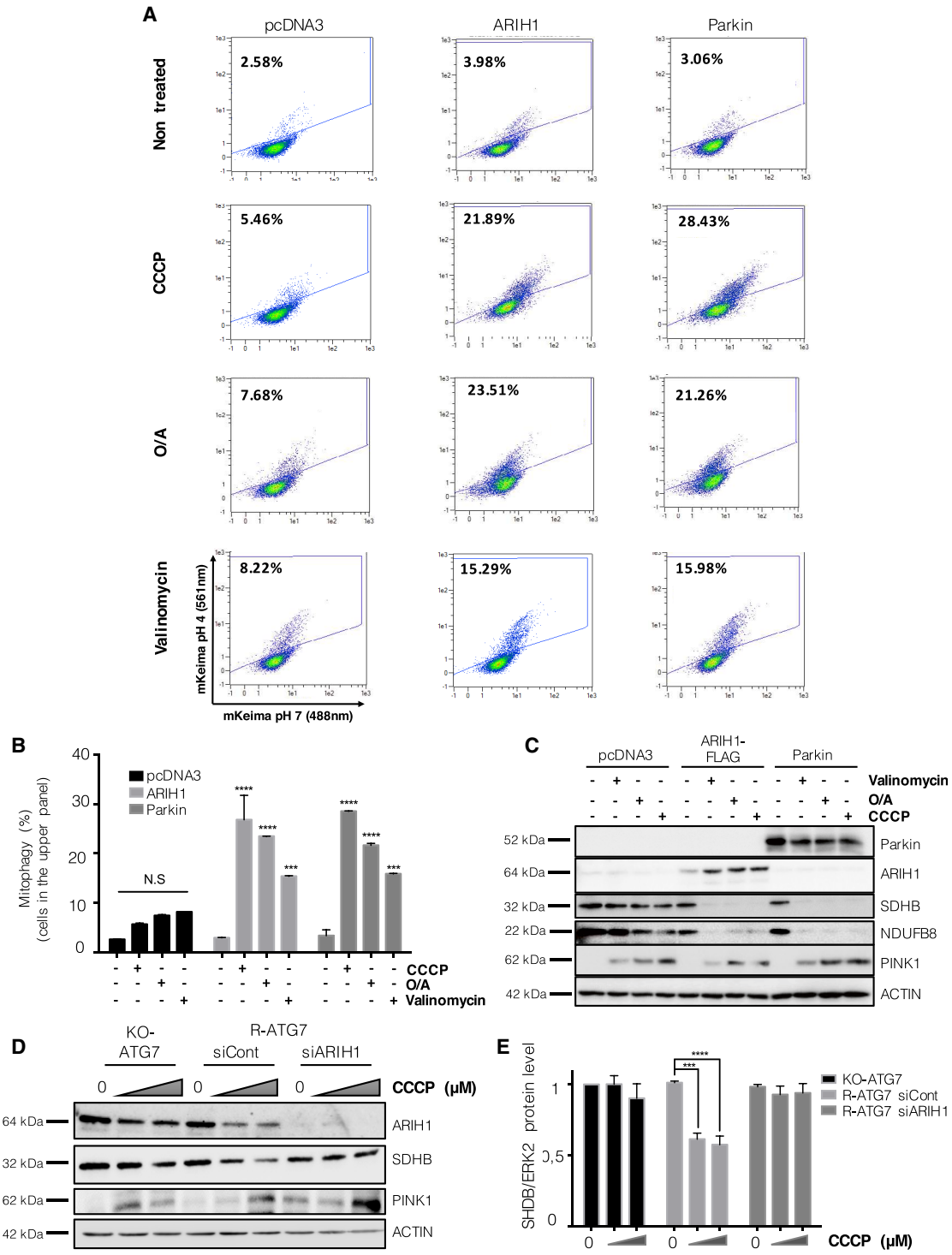


Figure 2. ARIH1 Removes Damaged Mitochondria via Autophagy

(A) HeLa cells overexpressing pcDNA3, ARIH1, or Parkin were transfected with m-Keima; treated with CCCP (10 μM), oligomycin/antimycin A (O/A; 25 nM and 250 nM, respectively), or valinomycin (10 nM) for 24 hr; and analyzed by flow cytometry. Green fluorescence of m-Keima reflects mitochondria in the cytosol (FL_{mito}, green), while red fluorescence reflects mitochondria in lysosomes (FL_{lyso}, red). The ratio of mitophagy is reflected by the percentage of cells in the top panel.

(B) Data are shown as the mean ± SEM of 3 independent experiments performed as in (A).

(legend continued on next page)

and 3E) in HeLa cells overexpressing ARIH1. Indeed, while ARIH1 decreased COX IV (Figures 3C and 3D) and increased conversion from green to red m-Keima following CCCP treatment (Figure 3E), both effects were blunted upon PINK1 KO. Equivalent results were obtained upon treatment with other mitochondria-damaging agents (Figure 3E).

It has been shown that during mitophagy, PINK1 phosphorylates Parkin and Ub on Ser[65]. We established here that in response to CCCP treatment, ARIH1 was phosphorylated on a Ser/Thr residue (Figure 3F), suggesting that phosphorylation by PINK1 is the first step in ARIH1-mediated mitophagy. Thus, ARIH1-dependent mitophagy requires PINK1 expression.

We then verified that the mitochondria of ARIH1-expressing HeLa cells were polyubiquitinated upon CCCP treatment as determined by the increase in Ub staining that co-localized with TOM20 staining on the mitochondria (Figure 4A). We also established that upon relocalization to the mitochondria, ARIH1 expression leads to TOM20 and MFN2 (mitofusin 2) degradation in a proteasomal-dependent manner, as MG132 could prevent it (Figure 4B).

We then used a mutant of ARIH1 with deletions in the RING type 1, IBR type, and RING type 2 domain (referred to as ΔARIH1). As previously shown, overexpression of full-length ARIH1 or Parkin resulted in mitophagy upon CCCP treatment, as determined by the conversion of m-Keima from green to red (Figure 4C) and the decrease in COX IV and SDHB expression (Figures 4D and 4E). In contrast, while ΔARIH1 was overexpressed to the same extent as full-length ARIH1, it did not lead to mitophagy (Figures 4C–4E) upon CCCP treatment.

We could therefore conclude that ARIH1 induces mitophagy by polyubiquitination of the damaged mitochondria, leading to its removal by the autophagic machinery.

ARIH1 Is Overexpressed in Cancer Cells and Is the Main Regulator of Mitophagy in These Cells

After establishing ARIH1 as a regulator of mitophagy, we assessed its expression in various cell lines and tissues (Figures 5A, 5B, and S4). ARIH1 mRNA was widely expressed in human cancer tissues and cancer cell lines, with strong expression in lung adenocarcinoma samples (Figures S4A and S4B). We then investigated ARIH1 protein expression in a panel of 9 different human cancer cell lines (Figure 5A). In contrast to Parkin, which was not expressed in any of the tumor cell lines tested (either at the protein or mRNA level; Figures 5A, 5B, and S4C), ARIH1 was endogenously expressed in several of them (Figures 5A and 5B), with the highest expression in lung cancer cell lines (A549 and H1975).

We then determined whether endogenous expression of ARIH1 could activate mitophagy upon mitochondrial depolarization. To accomplish this, A549 and H1975 cells were incubated with increasing amounts of a decoupling agent, and mitophagy was assessed by measuring COX IV expression. As shown in Figures 5C and 5D, COX IV expression was reduced upon decoupling of the mitochondria. This removal of damaged mitochondria was associated with an increase in autophagic markers (LC3 conversion and decrease in p62 expression; Figure 5E), altogether indicating that endogenous ARIH1 expression in lung cancer cell lines induced the removal of damaged mitochondria through mitophagy.

Importantly, we then demonstrated using three independent siRNAs that ARIH1 knockdown was sufficient to prevent CCCP-induced mitophagy as determined by the absence of the decrease in COX IV expression (Figure 6A). Equivalent results were obtained in H1975 cells following CCCP treatment (Figure S5A). To further support our observations, we performed a rescue experiment by knocking down endogenous ARIH1 expression in A549 cells, and we re-expressed an siRNA-resistant ARIH1-FLAG. As presented in Figures 6B, 6C, and S5B, mitophagy was not observed upon ARIH1 knockdown in the presence of CCCP or O/A, while ARIH1-FLAG expression restored it. We then measured mitophagy using m-Keima (as done previously) using a mitochondrial-damaging treatment (O/A). Mitophagy was blunted upon ARIH1 knockdown (Figure 6C), while it was observed when ARIH1-FLAG was expressed (Figure 6D).

We then deleted PINK1 expression using a CRISPR/Cas9 interference technique to validate the implication of this kinase in ARIH1-mediated mitophagy upon endogenous expression of this E3 ligase (Figures 6E and 6F). While mitochondrial network collapse around the perinuclear region and PINK1 induction (two early signs of mitophagy) could be observed in control cells, these markers were absent in A549 cells lacking PINK1. Similarly, COX IV reduction following CCCP treatment was not observed in cells lacking PINK1 (Figure 6F). We confirmed that the removal of damaged mitochondria in A549 cells was indeed mediated through mitophagy, as a cellular invalidation of ATG12 or ATG7 using the CRISPR/Cas9 technique (Wang et al., 2014) prevented CCCP-induced COX IV degradation and m-Keima conversion (Figures 6G–6I).

It was recently suggested that optineurin and NDP52 are key cargo adaptors for Parkin-mediated mitophagy (Heo et al., 2015; Lazarou et al., 2015; Richter et al., 2016). We therefore knocked down their expression using specific siRNA (Figures S5C and S5D) to evaluate their implication in ARIH1-mediated mitophagy. While respective protein expression was massively reduced in A549 cells, it did not prevent the COX IV decrease observed upon CCCP treatment, altogether indicating that neither optineurin nor NDP52 acts as a cargo adaptor for ARIH1-mediated mitophagy. MFN2 was recently suggested to be a mitochondrial receptor for Parkin that is required for mitophagy (Chen and Dorn, 2013). Using two independent

(C) Whole-cell lysates were treated as in (A) and analyzed for SDHB, NDUFB8, PINK1, Parkin, and ARIH1 expression by immunoblotting (actin was used as a loading control).
(D) Mitophagy was analyzed in MEF KO ATG7 cells and MEF KO cells reconstituted with ATG7-GFP (R-ATG7 MEFs) transfected with an siRNA control or an siRNA targeting ARIH1 by immunoblotting for SDHB or PINK1.
(E) Data are shown as the mean ± SEM of 3 independent experiments.
***$p < 0.001$, ****$p < 0.0001$ according to a two-way ANOVA.

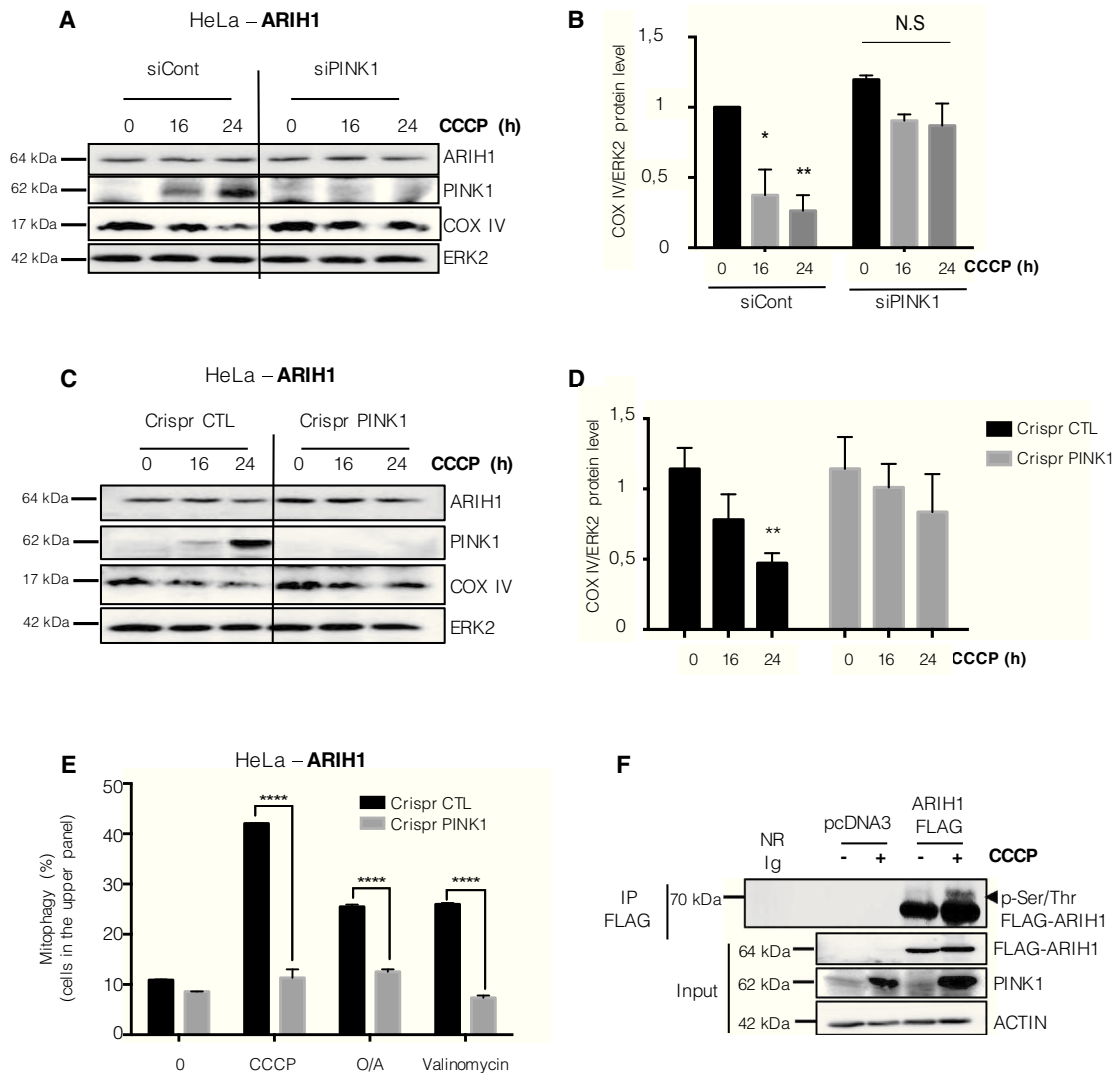


Figure 3. ARIH1-Dependent Mitophagy Requires PINK1

HeLa cells overexpressing ARIH1 were transfected with an siRNA control or an siRNA targeting PINK1 and treated with CCCP (10 μM) for the indicated times.

(A) Whole-cell lysates were analyzed for COX IV, PINK1, and ARIH1 expression by immunoblotting (ERK2 was used as a loading control).

(B) Data are shown as the mean ± SEM of 3 independent experiments.

(C–E) HeLa-expressing ARIH1 were transfected with a control construct (CRISPR CTL) or CRISPR/Cas9 construct in order to delete PINK1 and then treated with CCCP (10 μM) for the indicated times.

(C) Whole-cell lysates were analyzed for COX IV, PINK1, and ARIH1 expression by immunoblotting (ERK2 was used as a loading control).

(D) Data are shown as the mean ± SEM of 3 independent experiments.

(E) HeLa cells CRISPR CTL or CRISPR PINK1 ARIH1 were transfected with m-Keima; treated with CCCP (10 μM), oligomycin/antimycin A (O/A; 25 nM and 250 nM, respectively), or valinomycin (10 nM) for 24 hr; and analyzed by flow cytometry as in Figures 2A and 2B. Data are shown as the mean ± SEM of 3 independent experiments.

(F) HeLa cells were transfected with pcDNA3 or FLAG-ARIH1 plasmids. Cells were left untreated (left) or treated with CCCP (10 μM) for 4 hr (right). ARIH1 was immunoprecipitated (IP) with an anti-FLAG antibody followed by immunoblotting for phospho-Ser/Thr antibody. Actin was used as a loading control.

*p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 according to a two-way ANOVA. N.S: non-significant.

siRNAs, we established that MNF2 knockdown does not impair ARIH1-mediated mitophagy or sensitivity to cisplatin (Figures S5E–S5H).

Overall, we detected endogenous expression of ARIH1 in several cancer cell lines, including lung cancer cells, and established that ARIH1 is the main regulator of PINK1-dependent mitophagy upon mitochondrial damage.

ARIH1-Mediated Mitophagy Controls the Sensitivity of Lung Cancer Cells to Chemotherapy-Induced Death

After we identified ARIH1 as an endogenous regulator of mitophagy in lung cancer cells, we then decided to investigate its role in cell sensitivity or resistance to chemotherapy. We first investigated whether modulation of ARIH1 expression had an impact on cell survival. To accomplish this, HeLa cells expressing

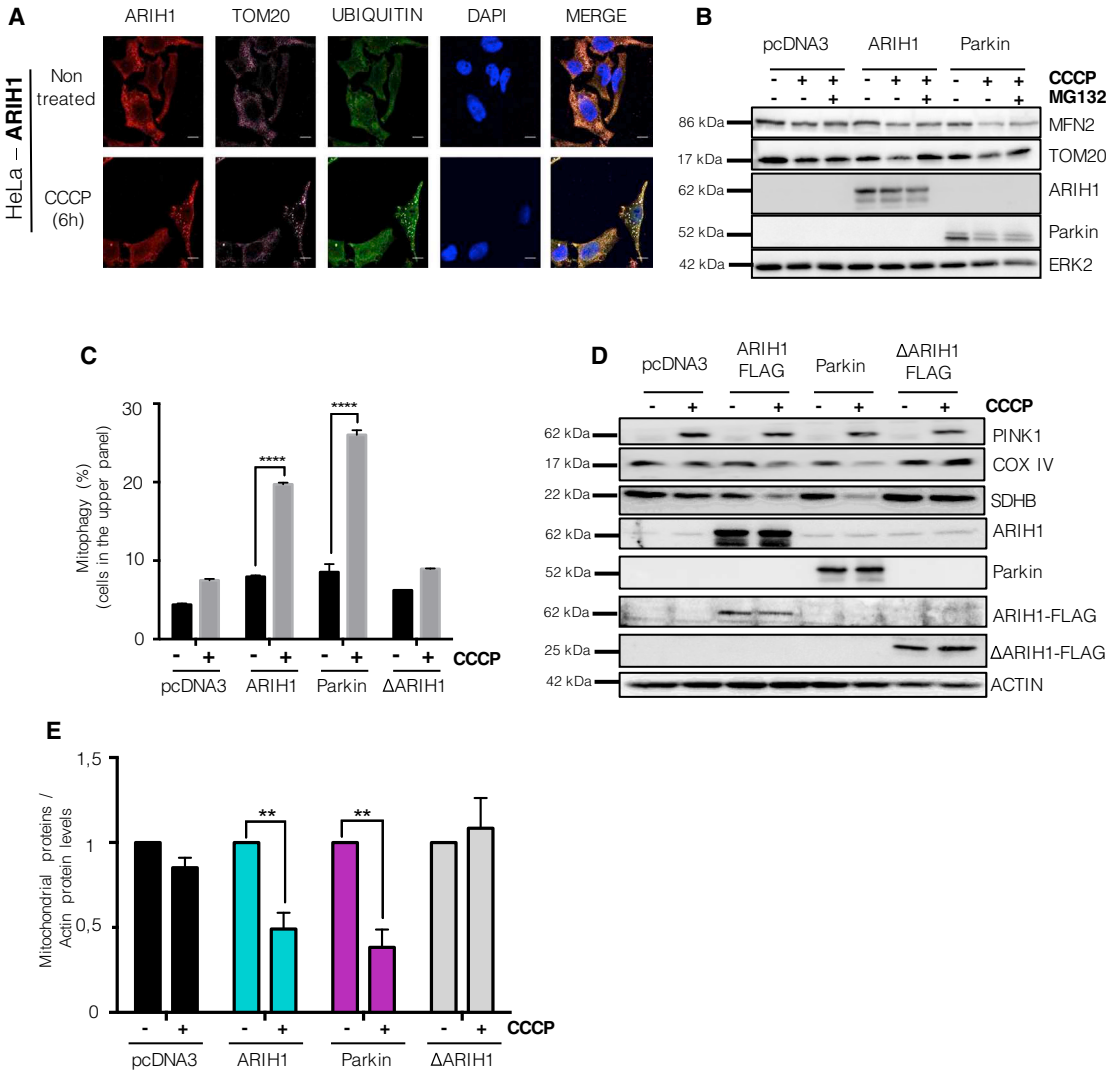


Figure 4. ARIH1-Dependent Mitophagy Requires Its Ub Ligase Activity

(A) ARIH1-overexpressing HeLa cells were treated with CCCP (10 μM) and co-immunostained for TOM20 (pink), Ub (green), and ARIH1 (red). Co-localization was analyzed by confocal microscopy (scale bar, 10 μm).

(B) HeLa cells were transiently transfected with a control vector (pcDNA$_3$), ARIH1, or Parkin and were treated with the mitochondrial uncoupling agent CCCP (10 μM) for 24 hr alone or with MG132 (10 μM). Whole-cell lysates were analyzed for TOM20, MFN2, PINK1, ARIH1, and Parkin expression by immunoblotting (ERK2 was used as a loading control).

(C) HeLa cells overexpressing pcDNA3, ARIH1, Parkin, or a truncated form of ARIH1 (ΔARIH1-FLAG) were transfected with m-Keima and treated with CCCP (10 μM) for 24 hr and analyzed by flow cytometry as in Figures 2A and 2B. Data are shown as the mean ± SEM of 3 independent experiments.

(D and E) HeLa cells were transiently transfected with a control vector (pcDNA$_3$), ARIH1-FLAG, Parkin, or a truncated form of ARIH1 (ΔARIH1-FLAG) and treated with the mitochondrial uncoupling agent CCCP (10 μM) for 24 hr.

(D) Whole-cell lysates were analyzed for COX IV, SDHB, PINK1, ARIH1, Parkin, and FLAG expression by immunoblotting (actin was used as a loading control).

(E) Data are shown as the mean ± SEM of 3 independent experiments.

**$p < 0.01$ and ****$p < 0.0001$ according to a two-way ANOVA.

ARIH1 were treated with CCCP, and we monitored their ability to grow as clones (clonogenic test). We observed that while only a few control HeLa cells could grow after CCCP treatment, the number of clones was significantly increased in cells expressing ARIH1 (Figure 7A), suggesting a protective effect of ARIH1 following mitochondrial damage.

We then verified that ARIH1 knockdown in A549 cells did not affect CCCP-induced mitochondrial depolarization (Figure S6A) or the ability of these cells to form colonies (Figure 7B). In contrast, knockdown of endogenous ARIH1 was sufficient to reduce the ability of A549 cells to survive following treatment with CCCP (Figure 7B). Indeed, two independent siRNAs targeting ARIH1 sensitized A549 cells to CCCP-induced apoptosis, as shown by an increase in PARP cleavage (Figure 7C), an increase in DEVDase activity (Figure 7D), and an increase in sub-G1 DNA content (Figure 7H), typical hallmarks of apoptosis. This

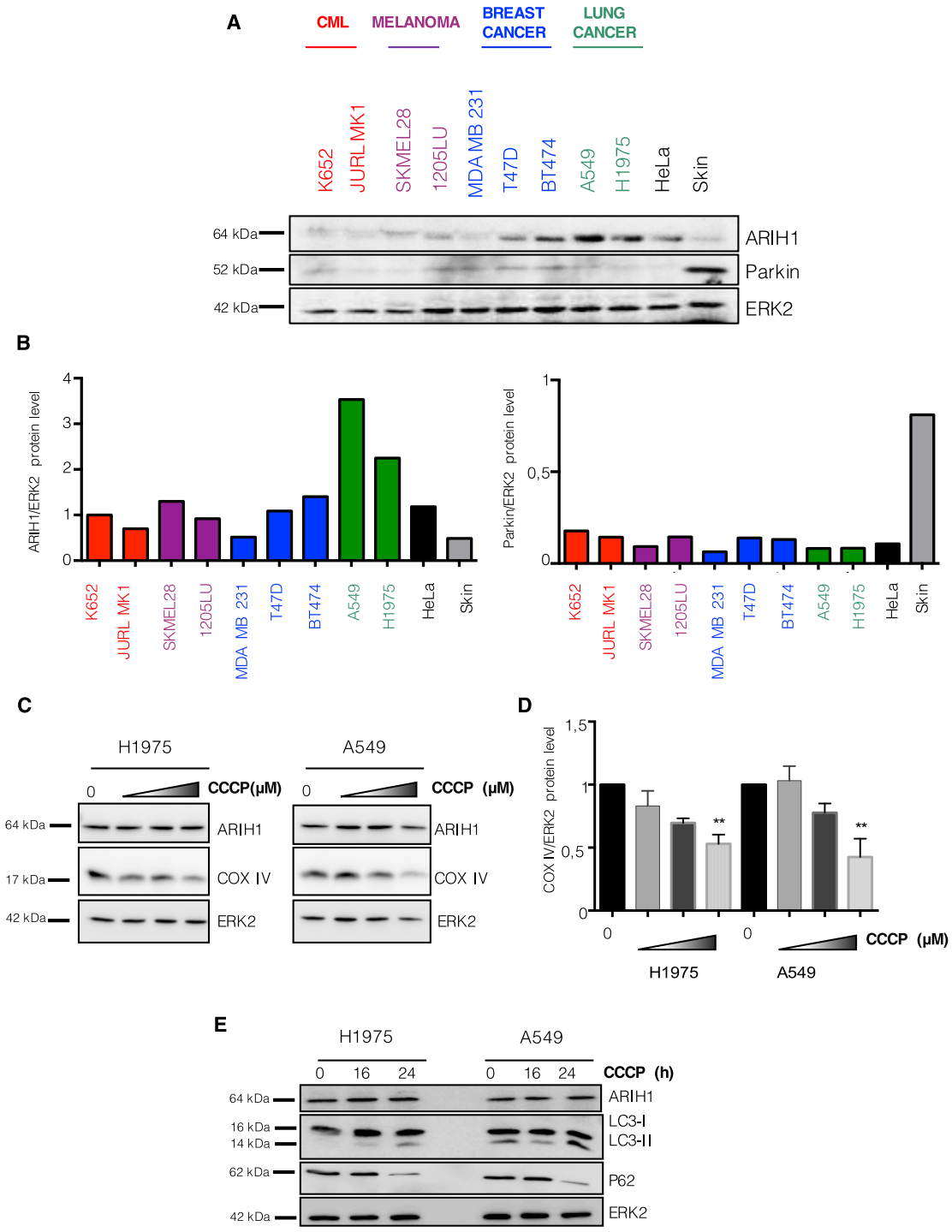


Figure 5. ARIH1 Is Overexpressed in Breast and Lung Cancer Cells

(A) Immunoblots of ARIH1 and Parkin in the indicated cancer cell lines and healthy skin sample (used as a positive control for endogenous Parkin expression). ERK2 was used as a loading control.

(B) The ratio of ARIH1 or Parkin to ERK2 expression (average of 2 independent experiments).

(C) A549 and H1975 lung cancer cell lines were treated with CCCP (from 1 to 10 μM) for 24 hr. The decrease in mitochondrial mass was analyzed by immunoblotting COX IV.

(legend continued on next page)

sensitization to CCCP-induced apoptosis following ARIH1 knockdown was also observed in H1975 cells (Figures S6B and S6C).

The alkylating agent cisplatin is a standard treatment for several cancers, including lung carcinomas. We therefore investigated whether endogenous ARIH1 expression could affect cisplatin-induced cell death. We selected a dose of cisplatin with a limited ability to prevent control A549 cell growth (transfected with a scramble siRNA). Strikingly, the same treatment substantially impaired the growth of ARIH1 knockdown A549 cells (Figure 7E) and sensitized these cells to apoptosis, as shown by the increase in PARP cleavage (Figure 7F), DEVDase activity (Figure 7G), and sub-G1 DNA content (Figure 7H). We confirmed that the decrease in mitochondrial potential ($\Delta\Psi m$) upon cisplatin treatment was not altered in ARIH1 knockdown cells, indicating that this E3 ligase acts downstream of the mitochondrial dysfunction (Figure S6D).

We demonstrated that ARIH1 functions both in mitophagy and in resistance to cisplatin. To determine whether ARIH1 promotes resistance to chemotherapy through mitophagy, we knocked out the molecular actors implicated in ARIH1-mediated mitophagy (PINK1, ATG7, and ATG12) in A549 cells. KO of those key proteins sensitized cells to various types of chemotherapy-induced death (Figures 7I, 7J, and S7A), suggesting that lung cancer cells use mitophagy as a defense mechanism against chemotherapy-induced cell death.

Finally, Parkin was recently suggested to regulate Bax levels and promote resistance to apoptosis independently of mitophagy (Cakir et al., 2017; Johnson et al., 2012). We did not observe any regulation of Bax or Bak levels upon ARIH1 modulation or PINK1 KO, suggesting that ARIH1-dependent control of cell death was not mediated by the regulation of the level of expression of those Bcl-2 members (Figures S6E–S6G). These results suggest that ARIH1-dependent mitophagy is protective in cancer cells.

DISCUSSION

The removal of dysfunctional mitochondria is required to maintain a healthy mitochondrial network and promote cell survival in response to certain stresses. How mitophagy promotes the turnover of damaged mitochondria that would otherwise injure the cell has not been fully elucidated. The most extensively characterized mitophagy regulators are Parkin/PINK1, BNIP3, and NIX (known as BNIP3L), which have non-overlapping roles in promoting autophagy (for review, see Chourasia et al., 2015). Importantly, in most cancers, BNIP3, NIX, and Parkin expression has been shown to be downregulated, indicating their role as tumor suppressors. Indeed, significant deletions of the BNIP3 locus at 10q26.3 were observed in half of the human tumor types, including lung carcinomas (Beroukhim et al., 2010). In addition, epigenetic silencing of BNIP3 expression as tumors progress to invasion and metastasis has been reported (Calvisi et al., 2007; Erkan et al., 2005). Similarly, Parkin (*PARK2*) maps to a common fragile site on human chromosome 6q25-q26 that is frequently deleted in cancers (Cesari et al., 2003). Therefore, until now the main regulators of mitophagy were considered as tumor suppressors and, therefore, the vast majority of the studies suggesting that the removal of damaged mitochondria could play a role in the survival of cancer cells following chemotherapeutic treatment could only be obtained after ectopic expression of those genes.

In sharp contrast, we identify here that the E3 ligase ARIH1 is regulator of PINK1-dependent mitophagy (Figures 1, 2, 3, and 4) that is overexpressed in several cancers, including lung adenocarcinomas (Figures 5, 6, and S4). We established that ARIH1-dependent control of mitophagy was indeed dependent on its Ub ligase activity (Figure 4). Importantly, we showed that ARIH1 overexpression is associated with resistance to chemotherapeutic-induced apoptosis (Figure 7). We also demonstrated that removal of ARIH1 or of key mitophagy or autophagy regulators sensitized tumor cells to chemotherapy-induced death (Figure 7), suggesting that mitophagy is protective in those cells, although we cannot formally exclude at this stage that other cellular functions of those proteins (which remain to be identified) are partially involved in the described effect.

Our results also suggest that ARIH1, as opposed to Parkin, BNIP3, or NIX, could be a predictive marker of chemotherapy. This notion is supported by the observation that lung adenocarcinoma patients with high levels of ARIH1 showed decreased survival after treatment (Figure S7B).

ARIH1 is a 557-amino-acid protein (64 kDa) distributed in the cytoplasm and the nuclei of cells (Figure 1A; Elmehdawi et al., 2013) that shares many structural and functional properties with Parkin (Parelkar et al., 2012). It is highly conserved, sharing 72% and 98% amino acid sequence identity with the *Drosophila* and mouse genes, respectively (Tan et al., 2000). Despite the widespread distribution of ARIH1 transcripts (Moynihan et al., 1999) and the lethality of KOs (Aguilera et al., 2000), its cellular functions are not well characterized. It was previously reported that ARIH1 levels were higher in cancer tissues than in normal tissues of the same origin (Elmehdawi et al., 2013), as opposed to the other known regulators of mitophagy. The same study reported that increased ARIH1 expression was associated with enhanced cell proliferation (Elmehdawi et al., 2013). Mechanistically, a yeast two-hybrid screen suggested an interaction between ARIH1 and the protein translation initiation factor eIF4E2 (Tan et al., 2003), suggesting a role in protein translation or RNA processing. It was later shown that this interaction with eIF4E2 was required for the protection of embryonic stem cells (ESCs) from DNA damage (von Stechow et al., 2015). However, whether this ARIH1-dependent control of mRNA translation arrest is required for the control of mitophagy in cancer cells is not known and will be the subject of further studies.

At the molecular level, it will be important to uncover how PINK1 activates ARIH1. Recently, ARIH1 was shown to be a

(D) Data are shown as the mean ± SEM of 3 independent experiments.
(E) A549 and H1975 lung cancer cell lines were treated with 10 μM CCCP for the indicated times, and autophagy induction was assessed by immunoblotting LC3 and P62. ERK2 was used as a loading control.
**$p < 0.01$ according to a two-way ANOVA.

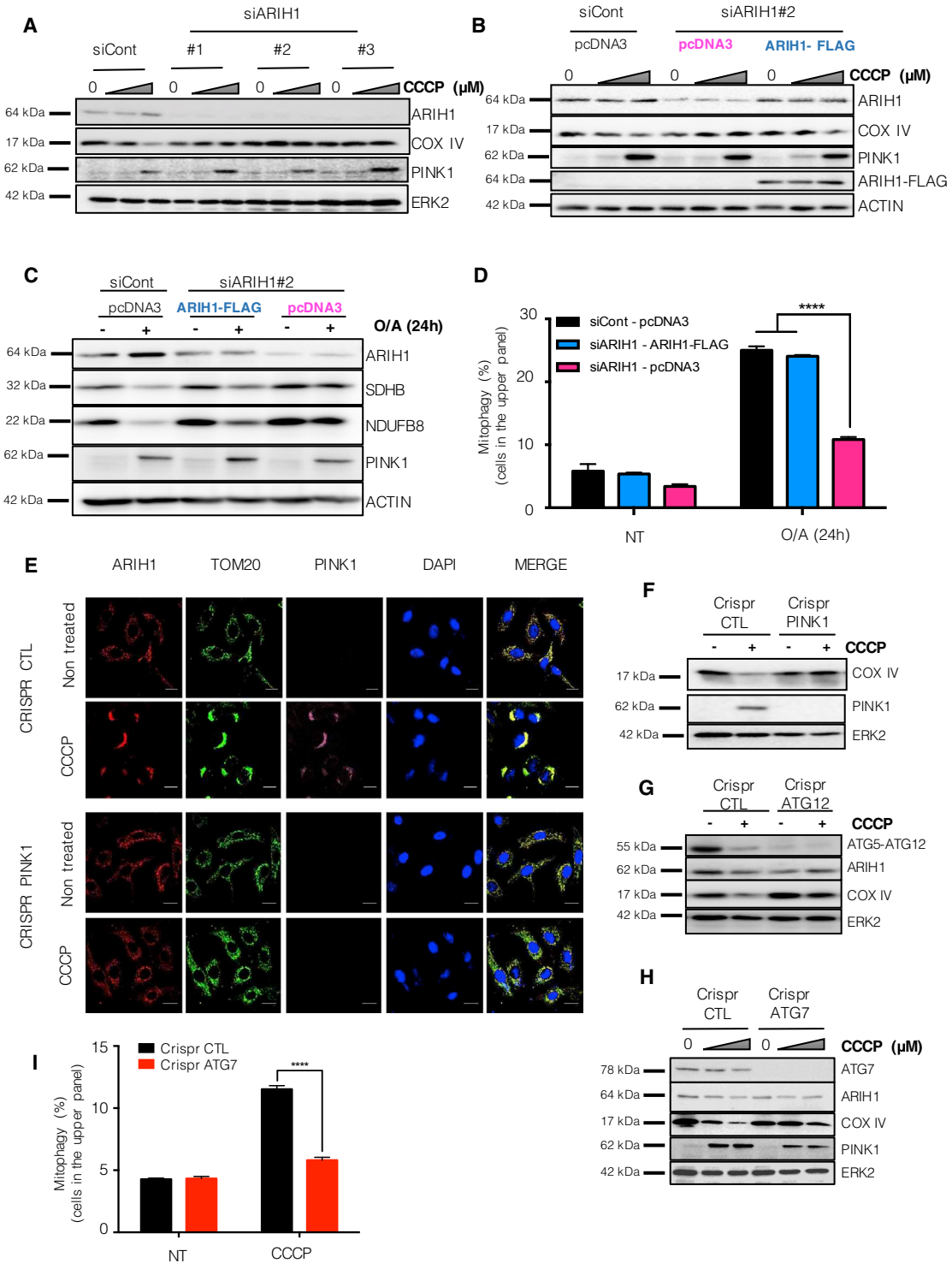


Figure 6. ARIH1 Induces Mitophagy in Lung Cancer Cells

A549 cells were transfected with the indicated siRNAs and treated with increasing amounts of CCCP (from 1 to 10 μM) for 24 hr.

(A) Whole-cell lysates were analyzed for COX IV, PINK1, and ARIH1 expression by immunoblotting (ERK2 was used as a loading control).

(B and C) A549 cells were transfected with the indicated siRNAs and then transfected with either an empty vector or FLAG-ARIH1 in order to rescue the knockdown of ARIH1 expression.

(B) Cells were treated with increasing amounts of CCCP (from 1 to 10 μM). Whole-cell lysates were analyzed for SDHB, NDUFB8, PINK1, and ARIH1 expression by immunoblotting (actin was used as a loading control).

(legend continued on next page)

new component of the cullin-RING E3 ligases (CRLs), which specifically mediate monoubiquitylation of several substrates (Scott et al., 2016). We therefore speculate that this novel role of ARIH1 could at least in part be involved in PINK1-mediated mitophagy, possibly through direct interaction with PINK1. PINK1 is a Ser/Thr kinase stabilized at the outer membrane of depolarized mitochondria, and it can phosphorylate Parkin in its UBL domain in order to overcome its autoinhibitory mechanism. Here, we show that ARIH1, after a brief exposure to CCCP, can also be phosphorylated on a Ser/Thr residue (Figure 3F). It is important to note that, like Parkin, ARIH1 also has an inhibitory (Ariadne) domain masking the RING type 2 domain containing catalytic activity. Furthermore, this Ariadne domain contains eight serine residues and two threonine residues that could potentially be phosphorylated by PINK1, leading to the unmasking of the ARIH1 catalytic site. Also, we established that ARIH1-mediated mitophagy and protection from chemotherapy-induced death was dependent on PINK1 expression, but not on NDP52, optineurin, MFN2, Bax, or Bak expression (Figure S5), suggesting that ARIH1 has a different set of targets than Parkin that remains to be identified.

Another open question is how ARIH1 is overexpressed in cancer cells. A screen of different public databases indicated that ARIH1 mRNA expression is upregulated in a wide variety of cancer tissues. As an example, a survey of Tumorscape (Broad Institute, Cambridge, MA, USA) (Beroukhim et al., 2010) indicated that ARIH1 was overexpressed in colorectal and medulloblastoma as well as in 21 out of 40 lung squamous carcinoma samples (tissues/cell lines) present in the database. These data suggest that transcriptional regulation of ARIH1 may be involved. However, the transcription factors involved have not been identified. Therefore, in addition to gene expression, the regulation of ARIH1 protein stability cannot be excluded at this stage.

The role of Parkin in the regulation of cell death is debated. While it is widely assumed to inhibit cell death, it was recently shown that several anti-apoptotic members of the Bcl-2 family could prevent Parkin translocation to the depolarized mitochondria and therefore regulate the onset of Parkin-dependent mitochondrial clearance (Hollville et al., 2014). It was then suggested that Parkin activation in response to mitochondrial dysfunction resulted in apoptosis by promoting the degradation of Mcl-1, a pro-survival gene of the Bcl-2 family (Carroll et al., 2014). These results suggest a close link between Parkin and the Bcl-2 family. However, as Parkin is rarely expressed in cancer cells, it would be interesting to investigate whether ARIH1 can interact with and control these pro-survival factors, as (1) ARIH1 was reported to limit protein translation, and (2) Mcl-1 has a short half-life and is strongly affected by a block in translation (Meynet et al., 2012, 2013; Pradelli et al., 2010).

Finally, it was recently suggested that PINK1 could induce mitophagy directly through phospho-Ub-mediated recruitment of autophagy receptors (Lazarou et al., 2015). While this interesting observation might be relevant to cells that do not express significant levels of Parkin or ARIH1, these results should be interpreted with caution in cells expressing ARIH1 (such as breast or lung cancer cell lines).

In conclusion, we show here that ARIH1 is a regulator of mitophagy in cancer cells that is involved in the protection of these cells from chemotherapy-induced death. This report challenges the view that the main regulators of mitophagy are tumor suppressors and suggests that ARIH1 may facilitate the removal of damaged mitochondria to promote tumor resistance to chemotherapy. While the association between Parkin genotype and cancer susceptibility is still under debate (Alcalay et al., 2012), our work indicates that ARIH1 is a potential therapeutic target and potentially a predictive marker of lung cancer sensitivity to chemotherapy.

EXPERIMENTAL PROCEDURES

Cell Death Measurement

To induce cell death, cells were treated with CCCP, cisplatin, or etoposide or irradiated with a UV lamp (254 nm) with the indicated doses. Cell death was analyzed either by DEVDase activity or DAPI staining. To assess DEVDase activity, cells were lysed in buffer (10 mM HEPES [pH 7.4], 150 mM NaCl, 5 mM EDTA, 1% NP40, 10 μg/mL aprotinin, 1 mM PMSF, and 10 μM leupeptin) 16 hr after treatment. Lysates were standardized for protein content and loaded into a black 96-well plate (CellStar) in the presence of 0.2 mmol/L of the caspase-3 substrate Ac-DEVD-AMC diluted in the following buffer: 50 mmol/L HEPES (pH 7.5), 150 mmol/L NaCl, 20 mmol/L EDTA, and 10 mmol/L DTT. Caspase activity was determined both with and without the presence of 1 μmol/L Ac-DEVD-CHO using a fluoroscan at 460 nm, and specific activity was expressed as the change in absorbance per minute per milligram protein.

In parallel, cell viability of the treated cells was assessed by looking at the plasma membrane permeabilization using DAPI staining and then analyzed by flow cytometry (Miltenyi Biotec).

For the cell cycle experiments, cells treated for 16 hr were permeabilized in 70% ethanol overnight at −20°C and washed with PBS. Cells were incubated with PBS, RNase (20 μg/mL), and propidium iodide (50 μg /mL, Sigma Aldrich) for 30 min at 4°C and then analyzed by flow cytometry (Miltenyi Biotec).

(C) Cells were treated with oligomycin/antimycin A (O/A; 25 nM and 250 nM, respectively) for 24 hr. Whole-cell lysates were analyzed for SDHB, NDUFB8, PINK1, and ARIH1 expression by immunoblotting (actin was used as a loading control).
(D) A549 were transfected with the indicated siRNAs and m-Keima, treated with O/A (25 nM and 250 nM, respectively) for 24 hr, and then analyzed by flow cytometry as in Figures 2A and 2B. Data are shown as the mean ± SEM of 3 independent experiments.
(E) A549 control cells (CRISPR CTL) and A549 cells invalidated for PINK1 (CRISPR PINK1) by the CRISPR/Cas9 technique were treated for 6 hr with CCCP (10 μM) and co-immunostained for TOM20 (green), PINK1 (pink), and ARIH1 (red). Co-localization was analyzed by confocal microscopy (scale bar, 10 μm).
(F) A549 CRISPR CTL and CRISPR PINK1 cells were treated for 24 hr with CCCP (10 μM). Whole-cell lysates were analyzed for COXIV, PINK1, and ARIH1 expression by immunoblotting (ERK2 was used as a loading control).
(G) A549 CRISPR CTL and CRISPR ATG12 cells were treated as described in (F).
(H) A549 CRISPR CTL and CRISPR ATG7 cells were treated for 24 hr with increasing amounts of CCCP (from 1 to 10 μM). Whole-cell lysates were analyzed for COX IV, PINK1, ATG7, and ARIH1 expression by immunoblotting (ERK2 was used as a loading control).
(I) A549 CRISPR CTL and CRISPR ATG7 were transfected with m-Keima, treated with CCCP (10 μM) for 24 hr, and analyzed by flow cytometry as in Figures 2A and 2B. Data are shown as the mean ± SEM of 3 independent experiments.
***$p < 0.001$ according to a two-way ANOVA.

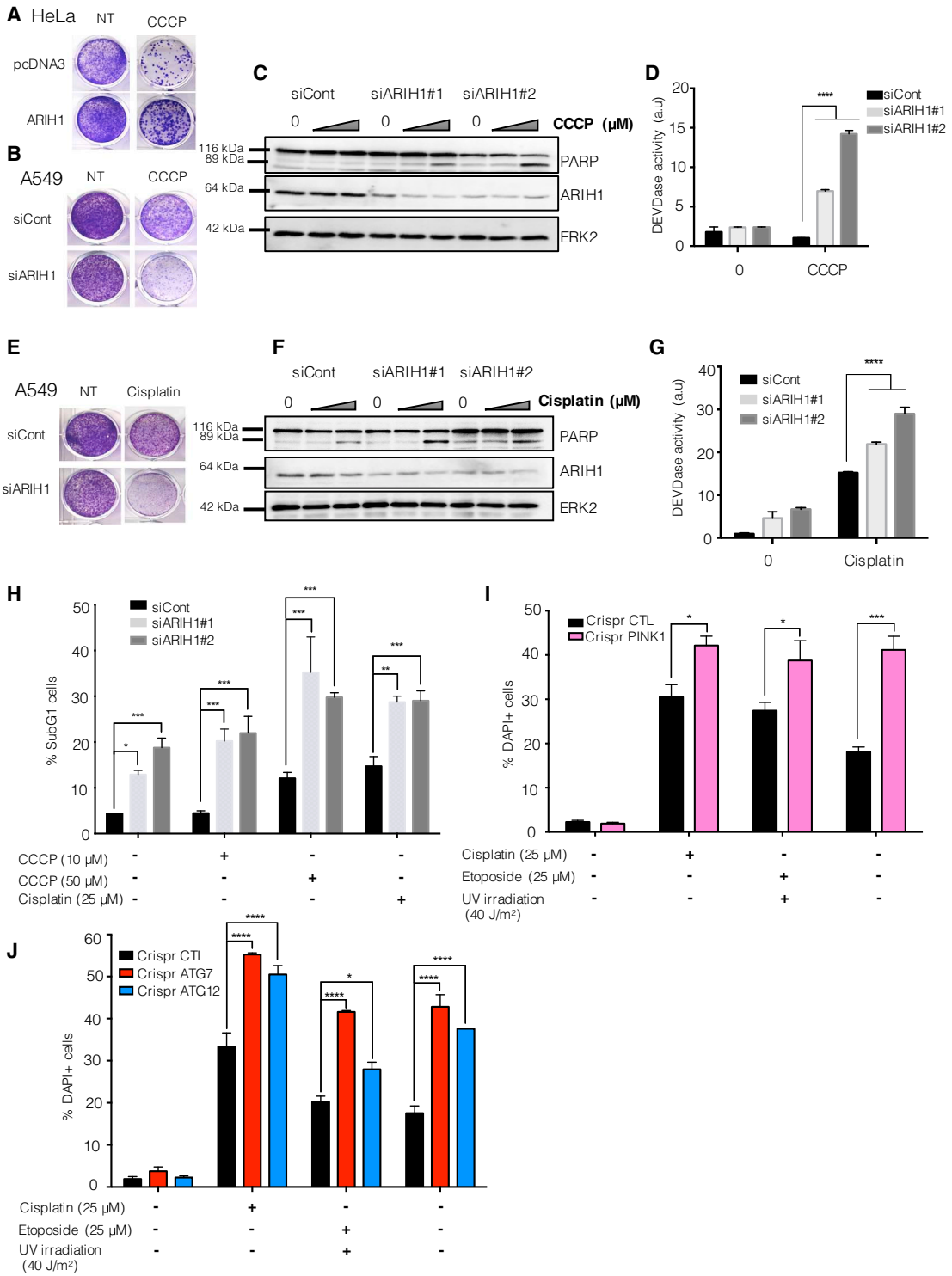


Figure 7. ARIH1 Protects Lung Cancer Cells from Cell Death

(A) Clonogenic assay of HeLa control cells (pcDNA$_3$) or cells overexpressing ARIH1, which were treated with CCCP (10 μM) for 6 hr. Pictures were taken 10 days after treatment.

(B) Clonogenic assay of A549 control cells (siCont) or ARIH1-silenced cells (siARIH1), which were treated with CCCP (10 μM) for 6 hr. Pictures were taken 5 days after treatment.

(C and D) A549 cells silenced for ARIH1 with two different siRNAs were treated with increasing concentrations of CCCP (from 1 to 10 μM). Apoptosis was analyzed by immunoblotting for PARP cleavage (C) and DEVDase activity (D).

(legend continued on next page)

Mito Keima Mitophagy Analysis

HeLa cells and A549 cells were transfected with m-Keima and then analyzed by flow cytometry (Miltenyi Biotec) as previously reported (Lazarou et al., 2015). Excitation 458 nm and emission >650 nm were used to detect m-Keima in mitochondria in the cytosol (FL_{mito}, green). Excitation 561 nm and emission >650 nm were used to detect mitochondria in lysosomes (FL_{lyso}, red).

Statistical Methods

Data are expressed as mean ± SEM. Differences in the calculated means between groups were assessed by two-way ANOVA.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and seven figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.08.087.

AUTHOR CONTRIBUTIONS

E.V. performed most experiments. E.P., C.R.-P., B.Z., J.P.B., R.M.R, J.C., and L.M. performed experiments. S.O., J.S.R., S.M., E.V., and S.W.G.T. contributed reagents and scientific input. E.V. and J.E.-R. designed research and interpreted data. J.E.-R. wrote the manuscript.

ACKNOWLEDGMENTS

We gratefully acknowledge the Centre Méditerranéen de Médecine Moléculaire microscopy facility. We thank Drs. Peyron, Imbert, Genin, Zhang, Ryan, and Rezzonico for providing reagents and Dr. Muqit for PINK KO MEFs. We thank Conseil Général des AM et de la région PACA et Corse for their financial support. This work was supported by the Fondation ARC (Association pour la Recherche sur le Cancer) and the Agence Nationale de la Recherche (LABEX SIGNALIFE ANR-11-LABX-0028-01). E.V. is supported by La Ligue contre le Cancer. C.R.-P. is supported by the Fondation ARC and J.C. by Foundation Tourre. L.M and S.O. were supported by la Fondation pour la Recherche Médicale (FRM).

Received: May 22, 2017
Revised: August 8, 2017
Accepted: August 25, 2017
Published: September 19, 2017

REFERENCES

Aguilera, M., Oliveros, M., Martínez-Padrón, M., Barbas, J.A., and Ferrús, A. (2000). Ariadne-1: a vital Drosophila gene is required in development and defines a new conserved family of ring-finger proteins. Genetics *155*, 1231–1244.

Alcalay, R.N., Clark, L.N., Marder, K.S., and Bradley, W.E. (2012). Lack of association between cancer history and PARKIN genotype: a family based study in PARKIN/Parkinson's families. Genes Chromosomes Cancer *51*, 1109–1113.

Beroukhim, R., Mermel, C.H., Porter, D., Wei, G., Raychaudhuri, S., Donovan, J., Barretina, J., Boehm, J.S., Dobson, J., Urashima, M., et al. (2010). The landscape of somatic copy-number alteration across human cancers. Nature *463*, 899–905.

Cakir, Z., Funk, K., Lauterwasser, J., Todt, F., Zerbes, R.M., Oelgeklaus, A., Tanaka, A., van der Laan, M., and Edlich, F. (2017). Parkin promotes proteasomal degradation of misregulated BAX. J. Cell Sci. Published online July 31, 2017. http://dx.doi.org/10.1242/jcs.200162.

Calvisi, D.F., Ladu, S., Gorden, A., Farina, M., Lee, J.S., Conner, E.A., Schroeder, I., Factor, V.M., and Thorgeirsson, S.S. (2007). Mechanistic and prognostic significance of aberrant methylation in the molecular pathogenesis of human hepatocellular carcinoma. J. Clin. Invest. *117*, 2713–2722.

Carroll, R.G., Hollville, E., and Martin, S.J. (2014). Parkin sensitizes toward apoptosis induced by mitochondrial depolarization through promoting degradation of Mcl-1. Cell Rep. *9*, 1538–1553.

Cesari, R., Martin, E.S., Calin, G.A., Pentimalli, F., Bichi, R., McAdams, H., Trapasso, F., Drusco, A., Shimizu, M., Masciullo, V., et al. (2003). Parkin, a gene implicated in autosomal recessive juvenile parkinsonism, is a candidate tumor suppressor gene on chromosome 6q25-q27. Proc. Natl. Acad. Sci. USA *100*, 5956–5961.

Chen, Y., and Dorn, G.W., 2nd. (2013). PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. Science *340*, 471–475.

Chourasia, A.H., Boland, M.L., and Macleod, K.F. (2015). Mitophagy and cancer. Cancer Metab. *3*, 4.

Clark, I.E., Dodson, M.W., Jiang, C., Cao, J.H., Huh, J.R., Seol, J.H., Yoo, S.J., Hay, B.A., and Guo, M. (2006). Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. Nature *441*, 1162–1166.

Elmehdawi, F., Wheway, G., Szymanska, K., Adams, M., High, A.S., Johnson, C.A., and Robinson, P.A. (2013). Human Homolog of Drosophila Ariadne (HHARI) is a marker of cellular proliferation associated with nuclear bodies. Exp. Cell Res. *319*, 161–172.

Erkan, M., Kleeff, J., Esposito, I., Giese, T., Ketterer, K., Büchler, M.W., Giese, N.A., and Friess, H. (2005). Loss of BNIP3 expression is a late event in pancreatic cancer contributing to chemoresistance and worsened prognosis. Oncogene *24*, 4421–4432.

Gong, Y., Zack, T.I., Morris, L.G., Lin, K., Hukkelhoven, E., Raheja, R., Tan, I.L., Turcan, S., Veeriah, S., Meng, S., et al. (2014). Pan-cancer genetic analysis identifies PARK2 as a master regulator of G1/S cyclins. Nat. Genet. *46*, 588–594.

Heo, J.M., Ordureau, A., Paulo, J.A., Rinehart, J., and Harper, J.W. (2015). The PINK1-PARKIN Mitochondrial Ubiquitylation Pathway Drives a Program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy. Mol. Cell *60*, 7–20.

Herhaus, L., and Dikic, I. (2015). Expanding the ubiquitin code through post-translational modification. EMBO Rep. *16*, 1071–1083.

Hollville, E., Carroll, R.G., Cullen, S.P., and Martin, S.J. (2014). Bcl-2 family proteins participate in mitochondrial quality control by regulating Parkin/PINK1-dependent mitophagy. Mol. Cell *55*, 451–466.

Johnson, B.N., Berger, A.K., Cortese, G.P., and Lavoie, M.J. (2012). The ubiquitin E3 ligase parkin regulates the proapoptotic function of Bax. Proc. Natl. Acad. Sci. USA *109*, 6283–6288.

Kane, L.A., Lazarou, M., Fogel, A.I., Li, Y., Yamano, K., Sarraf, S.A., Banerjee, S., and Youle, R.J. (2014). PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. J. Cell Biol. *205*, 143–153.

Katayama, H., Kogure, T., Mizushima, N., Yoshimori, T., and Miyawaki, A. (2011). A sensitive and quantitative technique for detecting autophagic events based on lysosomal delivery. Chem. Biol. *18*, 1042–1052.

(E) Clonogenic assay of A549 control cells (siCont) or cells silenced for ARIH1 (siARIH1) and treated with cisplatin (25 μM) for 6 hr. Pictures were taken 5 days after treatment.
(F and G) A549 cells silenced for ARIH1 with two different siRNAs were treated with increasing concentrations of cisplatin. Apoptosis was analyzed by immunoblotting for PARP cleavage (F) and DEVDase activity (G).
(H) A549 cells silenced for ARIH1 were treated for 16 hr with the indicated amounts of CCCP or cisplatin (25 μM), and apoptosis was assessed using propidium iodide staining and flow cytometry measuring sub-G1 DNA fragmentation. Data are shown as the mean ± SEM of 3 independent experiments.
(I) A549 CRISPR CTL and CRISPR PINK1 cells were treated for 48 hr with cisplatin (25 μM) or etoposide (25 μM) or irradiated with UV (40 J/m^2). Plasma membrane permeabilization (i.e., cell death) was assessed using DAPI staining and analyzed by flow cytometry. Data are shown as the mean ± SEM of 3 independent experiments.
(J) A549 CRISPR CTL, A549 CRISPR ATG7, and A549 CRISPR ATG12 were treated as described in (I).

Kazlauskaite, A., Martínez-Torres, R.J., Wilkie, S., Kumar, A., Peltier, J., Gonzalez, A., Johnson, C., Zhang, J., Hope, A.G., Peggie, M., et al. (2015). Binding to serine 65-phosphorylated ubiquitin primes Parkin for optimal PINK1-dependent phosphorylation and activation. EMBO Rep. *16*, 939–954.

Kerscher, O., Felberbaum, R., and Hochstrasser, M. (2006). Modification of proteins by ubiquitin and ubiquitin-like proteins. Annu. Rev. Cell Dev. Biol. *22*, 159–180.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature *392*, 605–608.

Koyano, F., Okatsu, K., Kosako, H., Tamura, Y., Go, E., Kimura, M., Kimura, Y., Tsuchiya, H., Yoshihara, H., Hirokawa, T., et al. (2014). Ubiquitin is phosphorylated by PINK1 to activate parkin. Nature *510*, 162–166.

Kulathu, Y., and Komander, D. (2012). Atypical ubiquitylation: the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. Nat. Rev. Mol. Cell Biol. *13*, 508–523.

Lazarou, M., Sliter, D.A., Kane, L.A., Sarraf, S.A., Wang, C., Burman, J.L., Sideris, D.P., Fogel, A.I., and Youle, R.J. (2015). The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature *524*, 309–314.

Meynet, O., Bénéteau, M., Jacquin, M.A., Pradelli, L.A., Cornille, A., Carles, M., and Ricci, J.E. (2012). Glycolysis inhibition targets Mcl-1 to restore sensitivity of lymphoma cells to ABT-737-induced apoptosis. Leukemia *26*, 1145–1147.

Meynet, O., Zunino, B., Happo, L., Pradelli, L.A., Chiche, J., Jacquin, M.A., Mondragón, L., Tanti, J.F., Taillan, B., Garnier, G., et al. (2013). Caloric restriction modulates Mcl-1 expression and sensitizes lymphomas to BH3 mimetic in mice. Blood *122*, 2402–2411.

Moynihan, T.P., Ardley, H.C., Nuber, U., Rose, S.A., Jones, P.F., Markham, A.F., Scheffner, M., and Robinson, P.A. (1999). The ubiquitin-conjugating enzymes UbcH7 and UbcH8 interact with RING finger/IBR motif-containing domains of HHARI and H7-AP1. J. Biol. Chem. *274*, 30963–30968.

Nagy, V., and Dikic, I. (2010). Ubiquitin ligase complexes: from substrate selectivity to conjugational specificity. Biol. Chem. *391*, 163–169.

Narendra, D., Tanaka, A., Suen, D.F., and Youle, R.J. (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J. Cell Biol. *183*, 795–803.

Narendra, D., Kane, L.A., Hauser, D.N., Fearnley, I.M., and Youle, R.J. (2010). p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. Autophagy *6*, 1090–1106.

Okatsu, K., Saisho, K., Shimanuki, M., Nakada, K., Shitara, H., Sou, Y.S., Kimura, M., Sato, S., Hattori, N., Komatsu, M., et al. (2010). p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. Genes Cells *15*, 887–900.

Parelkar, S.S., Cadena, J.G., Kim, C., Wang, Z., Sugal, R., Bentley, B., Moral, L., Ardley, H.C., and Schwartz, L.M. (2012). The parkin-like human homolog of Drosophila ariadne-1 (HHARI) can induce aggresome formation in mammalian cells and is immunologically detectable in Lewy bodies. J. Mol. Neurosci. *46*, 109–121.

Park, J., Lee, S.B., Lee, S., Kim, Y., Song, S., Kim, S., Bae, E., Kim, J., Shong, M., Kim, J.M., and Chung, J. (2006). Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. Nature *441*, 1157–1161.

Pradelli, L.A., Bénéteau, M., Chauvin, C., Jacquin, M.A., Marchetti, S., Muñoz-Pinedo, C., Auberger, P., Pende, M., and Ricci, J.E. (2010). Glycolysis inhibition sensitizes tumor cells to death receptors-induced apoptosis by AMP kinase activation leading to Mcl-1 block in translation. Oncogene *29*, 1641–1652.

Richter, B., Sliter, D.A., Herhaus, L., Stolz, A., Wang, C., Beli, P., Zaffagnini, G., Wild, P., Martens, S., Wagner, S.A., et al. (2016). Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. Proc. Natl. Acad. Sci. USA *113*, 4039–4044.

Scott, D.C., Rhee, D.Y., Duda, D.M., Kelsall, I.R., Olszewski, J.L., Paulo, J.A., de Jong, A., Ovaa, H., Alpi, A.F., Harper, J.W., et al. (2016). Two distinct types of E3 ligases work in unison to regulate substrate ubiquitylation. Cell *166*, 1198–1214.

Shimura, H., Hattori, N., Kubo, Si., Mizuno, Y., Asakawa, S., Minoshima, S., Shimizu, N., Iwai, K., Chiba, T., Tanaka, K., and Suzuki, T. (2000). Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat. Genet. *25*, 302–305.

Spratt, D.E., Walden, H., and Shaw, G.S. (2014). RBR E3 ubiquitin ligases: new structures, new insights, new questions. Biochem. J. *458*, 421–437.

Taherbhoy, A.M., Tait, S.W., Kaiser, S.E., Williams, A.H., Deng, A., Nourse, A., Hammel, M., Kurinov, I., Rock, C.O., Green, D.R., and Schulman, B.A. (2011). Atg8 transfer from Atg7 to Atg3: a distinctive E1-E2 architecture and mechanism in the autophagy pathway. Mol. Cell *44*, 451–461.

Tan, N.G., Ardley, H.C., Rose, S.A., Leek, J.P., Markham, A.F., and Robinson, P.A. (2000). Characterisation of the human and mouse orthologues of the Drosophila ariadne gene. Cytogenet. Cell Genet. *90*, 242–245.

Tan, N.G., Ardley, H.C., Scott, G.B., Rose, S.A., Markham, A.F., and Robinson, P.A. (2003). Human homologue of ariadne promotes the ubiquitylation of translation initiation factor 4E homologous protein, 4EHP. FEBS Lett. *554*, 501–504.

Veeriah, S., Morris, L., Solit, D., and Chan, T.A. (2010a). The familial Parkinson disease gene PARK2 is a multisite tumor suppressor on chromosome 6q25.2-27 that regulates cyclin E. Cell Cycle *9*, 1451–1452.

Veeriah, S., Taylor, B.S., Meng, S., Fang, F., Yilmaz, E., Vivanco, I., Janakiraman, M., Schultz, N., Hanrahan, A.J., Pao, W., et al. (2010b). Somatic mutations of the Parkinson's disease-associated gene PARK2 in glioblastoma and other human malignancies. Nat. Genet. *42*, 77–82.

Villa, E., and Ricci, J.E. (2016). How does metabolism affect cell death in cancer? FEBS J. *283*, 2653–2660.

von Stechow, L., Typas, D., Carreras Puigvert, J., Oort, L., Siddappa, R., Pines, A., Vrieling, H., van de Water, B., Mullenders, L.H., and Danen, E.H. (2015). The E3 ubiquitin ligase ARIH1 protects against genotoxic stress by initiating a 4EHP-mediated mRNA translation arrest. Mol. Cell. Biol. *35*, 1254–1268.

Wallace, D.C. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu. Rev. Genet. *39*, 359–407.

Wang, T., Wei, J.J., Sabatini, D.M., and Lander, E.S. (2014). Genetic screens in human cells using the CRISPR-Cas9 system. Science *343*, 80–84.

Wauer, T., Simicek, M., Schubert, A., and Komander, D. (2015). Mechanism of phospho-ubiquitin-induced PARKIN activation. Nature *524*, 370–374.

Wei, H., Liu, L., and Chen, Q. (2015). Selective removal of mitochondria via mitophagy: distinct pathways for different mitochondrial stresses. Biochim. Biophys. Acta *1853* (*10 Pt B*), 2784–2790.

Wenzel, D.M., Lissounov, A., Brzovic, P.S., and Klevit, R.E. (2011). UBCH7 reactivity profile reveals parkin and HHARI to be RING/HECT hybrids. Nature *474*, 105–108.

Xu, L., Lin, D.C., Yin, D., and Koeffler, H.P. (2014). An emerging role of PARK2 in cancer. J. Mol. Med. (Berl.) *92*, 31–42.

5. Article 5:

GAPDH overexpression in the T cell lineage promotes angioimmunoblastic T cell lymphoma through an NF-κB dependent mechanism

Laura Mondragón[1,*], Rana Mhaidly[1,*], Gian Marco De Donatis[1], Marie Tosolini[2], Pascal Dao[3], Anthony R. Martin[3], Caroline Pons[1], Johanna Chiche[1], Marie Jacquin[1], Véronique Imbert[1], Emma Proïcs[1], Laurent Boyer[1], Patrick Munro[1], Frédéric Luciano[1], Jaap G. Neels[1,] Frédéric Coutant[4,5], Nicole Fabien[4], Laura Sormani1,Camila Rubio-Patiño[1], Jozef P. Bossowski[1], Florian Muller[1], Sandrine Marchetti[1], Elodie Villa[1], Jean-François Peyron[1], Laurent Genestier[6], Rachid Benhida[3], Jean-Jacques Fournié[7], Thierry Passeron[1,8], Jean-Ehrland Ricci[1,10] and Els Verhoeyen[1, 9,10,11].

[1]Université Côte d'Azur, INSERM, C3M, 06204 Nice, France.

[2]Pôle Technologique du CRCT – Plateau Bioinformatique INSERM-UMR1037

[3]Institut de Chimie de Nice UMR UNS-CNRS 7272, Université Nice Sophia Antipolis, Parc Valrose, 06108 Nice, France

[4]Immunology Department, Lyon-Sud Hospital, Hospices Civils de Lyon, Pierre-Bénite, France

[5]Immunogenomics and Inflammation Research Unit EA 4130, University of Lyon, Edouard Herriot Hospital, Lyon, France

[6]CRCI ; INSERMU1052-CNRS UMR5286 ; Centre Léon Bérard ; Faculté de Medecine Lyon Sud, Université Claude Bernard Lyon I, 69921 Oullins Cedex, France.

[7]CRCT; INSERM U1037-Université Paul Sabatier-CNRS ERL5294, Université de Toulouse; Laboratoire d'Excellence TOUCAN; Programme Hospitalo-Universitaire en Cancérologie CAPTOR, Toulouse, France; IUCT, 31037 Toulouse, France

[8]Université Côte d'Azur. Centre Hospitalier Universitaire de Nice. Department of Dermatology. 06204 Nice, France
[9]CIRI, Université de Lyon; INSERM U1111; ENS de Lyon; Université Lyon1 ; CNRS, UMR 5308, 69007 Lyon, France
*equal contribution
[10]**Corresponding authors**: Els Verhoeyen (els.verhoeyen@unice.fr/els.verhoeyen@ens-lyon.fr) and Jean-Ehrland Ricci (ricci@unice.fr)
[11]**Lead contact**: Els Verhoeyen, PhD: Inserm U1065, équipe 3, 151 route de St Antoine de Ginestière, BP 23194, 06204 Nice Cedex 03, France. Phone: +33 4 89 06 43 01; Fax +33 4 89 06 42 21, email: els.verhoeyen@unice.fr/els.verhoeyen@ens-lyon.fr

Summary

Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) is emerging as a key player in T-cell development, function and cancer. Here we investigated the role of GAPDH in T-cell development/function by overexpressing GAPDH in the T-cell lineage. Aged mice developed: 1) splenomegaly, 2) enlarged lymph nodes, 3) lymphocyte-infiltrations in the liver and bone marrow. All showed an increase of strongly proliferating and clonal Tfh $CXCR5^{+}PD1^{high}CD4^{+}$-T cells associated with germinal center B cells and inflammatory cytokine-release. Gene-set-expression-analysis confirmed that this lymphoma was equivalent to human angioimmunoblastic T-cell lymphoma (AITL). Mechanistically, GAPDH induced NF-κB pathway in the murine AITL *in vivo* inhibition of NF-κB combined with anti-PD1 increased mice survival and cancer immune response. GAPDH-dependent modulation of NF-κB in T-cells allowed to model AITL-disease and evaluate treatments.

This article is under revision in Cancer Cell.

6. Article 6:

Obacz, J., Avril, T., Rubio-Patino, C., **Bossowski, J. P**., Igbaria, A., Ricci, J. E., & Chevet, E. (2017). Regulation of tumor-stroma interactions by the unfolded protein response. FEBS J. doi:10.1111/febs.14359

The FEBS Journal

FEBS PRESS science publishing by scientists

REVIEW ARTICLE

Regulation of tumor–stroma interactions by the unfolded protein response

Joanna Obacz[1,2], Tony Avril[1,2], Camila Rubio-Patiño[3], Jozef P. Bossowski[3], Aeid Igbaria[4], Jean-Ehrland Ricci[3] and Eric Chevet[1,2]

1 Inserm U1242 'Chemistry, Oncogenesis, Stress & Signaling', Université de Rennes, Rennes, France
2 Centre de Lutte Contre le Cancer Eugene Marquis, Rennes, France
3 INSERM, C3M, Université Côte d'Azur, Nice, France
4 Department of Medicine, University of California, San Francisco, San Francisco, CA, USA

Keywords
ER stress; immunogenic cell death; inflammation; tumor microenvironment; unfolded protein response

Correspondence
E. Chevet, Inserm U1242 'Chemistry, Oncogenesis, Stress & Signaling,' Université de Rennes, Rennes, France
Fax: +33 299253164
Tel: +33 621707544
E-mail: eric.chevet@inserm.fr

(Received 16 October 2017, revised 16 November 2017, accepted 7 December 2017)

doi:10.1111/febs.14359

The unfolded protein response (UPR) is a conserved adaptive pathway that helps cells cope with the protein misfolding burden within the endoplasmic reticulum (ER). Imbalance between protein folding demand and capacity in the ER leads to a situation called ER stress that is often observed in highly proliferative and secretory tumor cells. As such, activation of the UPR signaling has emerged as a key adaptive mechanism promoting cancer progression. It is becoming widely acknowledged that, in addition to its intrinsic effect on tumor biology, the UPR can also regulate tumor microenvironment. In this review, we discuss how the UPR coordinates the crosstalk between tumor and stromal cells, such as endothelial cells, normal parenchymal cells, and immune cells. In addition, we further describe the involvement of ER stress signaling in the response to current treatments as well as its impact on antitumor immunity mainly driven by immunogenic cell death. Finally, in this context, we discuss the relevance of targeting ER stress/UPR signaling as a potential anticancer approach.

The UPR is an adaptive mechanism in cancer cells

Excessive endoplasmic reticulum (ER) stress is emerging as a hallmark of solid tumors. Cancer cells due to their high proliferative and secretory demands are at risk of the accumulation of improperly folded proteins in the ER lumen, which perturb the protein homeostasis (referred to as proteostasis) [1]. In addition, tumor cells are constantly exposed to the microenvironmental pressure such as hypoxia, glucose shortage, oxidative

Abbreviations
ATF6, activating transcription factor 6; CAFs, cancer-associated fibroblasts; CALR, calreticulin; CRYAB, chaperone αB-crystallin; CX, connexin; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; DLBCL, diffuse large B-cell lymphoma; ECs, endothelial cells; EMT, epithelial-to-mesenchymal transition; ERAD, ER-associated degradation; ER, endoplasmic reticulum; GADD34, growth arrest and DNA damage 34; GCB, germinal center B-cell; GJIC, gap junctional intercellular communication; GRP78, ER chaperone glucose-regulated protein 78; HSPs, heat-shock proteins; ICD, immunogenic cell death; IRE1α, inositol requiring enzyme 1 alpha; IRS, integrated stress response; MCP-1, monocyte chemoattractant protein-1; MEF, mouse embryonic fibroblast; MKC, ManKindCorp; MM, multiple myeloma; PERK, protein kinase PKR-like ER kinase; PRRs, pattern recognition receptors; RIDD, regulated IRE1-dependent decay; RIG-I, retinoic acid-inducible gene-I; ROS, reactive oxygen species; TERS, transmissible ER stress; TILs, tumor-infiltrating lymphocytes; TLR2, toll-like receptor 2; TME, tumor microenvironment; uPCA, urokinase plasminogen activator; UPR, unfolded protein response; XBP1, X-box binding protein-1.

stress, or low pH, all known to cause ER stress [2]. To cope with those challenges and restore proteostasis, cells activate the evolutionary conserved adaptive pathway known as unfolded protein response (UPR) by the coordinated action of three ER transmembrane proteins, namely the activating transcription factor 6 (ATF6), the protein kinase PKR-like ER kinase (PERK), and the inositol requiring enzyme 1 alpha (IRE1α, referred to as IRE1 hereafter) [2–4]. The current dogma in mammalian cells indicates that under non-stressed conditions, the ER chaperone glucose-regulated protein 78 (GRP78, also known as BiP) constitutively binds to the luminal domain of the three sensors precluding their activation. However, upon accumulation of unfolded/misfolded proteins in the ER, GRP78 dissociates from those complexes consequently triggering the UPR cascade [4]. It was also proposed that direct association of unfolded proteins to yeast and mammalian IRE1 could induce its activation through conformational change [5,6]. Upon ER stress, ATF6 is exported from the ER to the Golgi apparatus, where it is activated by the SP1- and SP2-medited proteolytic cleavage, which releases the cytosolic fragment of the protein, ATF6f [7,8]. The latter is further translocated to the nucleus to regulate the transcription of genes involved in ER-associated degradation (ERAD) and protein folding [9]. To reduce the protein misfolding burden in the ER, activated PERK phosphorylates the eukaryotic translation initiation factor eIF2α at serine 51, hence attenuating global protein synthesis [7,10]. This mechanism also allows the translational activation of the transcription factor ATF4, which controls the expression of genes impacting on amino acid metabolism, antioxidant response, autophagy, apoptosis, and protein folding [2,7,11]. Finally, in response to ER stress, IRE1, which harbors serine/threonine kinase and endoribonuclease (RNase) activities, dimerizes/oligomerizes and autotransphosphorylates. Active IRE1 catalyzes the unconventional splicing of X-box binding protein-1 (XBP1) mRNA and together with RTCB ligase yields an active transcription factor XBP1s. Consequently, XBP1 modulates the expression of genes involved in glycosylation, ERAD, protein folding, and lipid synthesis [4,12]. IRE1 endoribonuclease activity also targets other mRNAs and micro-RNAs in a process called regulated IRE1-dependent decay (RIDD), which controls cell fate under ER stress conditions [13]. Finally, IRE1 activates the ASK1/JNK1 signaling pathway through the recruitment of TRAF2 to IRE1 [14]. Depending on the time and duration of the ER stress, each arm of the UPR can either trigger the adaptive response to alleviate the ER stress or activate the pro-death signals when ER stress cannot be resolved (terminal UPR; reviewed in [15]). Briefly, the adaptive UPR relies on the activation of among others $p58^{IPK}$, chaperones, foldases, and antioxidant enzymes as well as inhibition of the pro-apoptotic CHOP [15]. Pro-death UPR, however, engages JNK- and NFκB-dependent activation of pro-apoptotic BCL-2 family proteins, induction of CHOP, and inhibition of anti-apoptotic miR-106b-25 [15].

Activation of the UPR in cancer

The overexpression of UPR sensors has been reported in a large number of human cancers including that of breast, brain, gastrointestinal tract, liver, kidney, pancreas, lung, and prostate [8]. In addition, elevated level of the main UPR regulator GRP78 is often found in tumor tissues [16] and is associated with metastasis, poor prognosis, and resistance to treatment [17–19]. The UPR involvement in cancer initiation and cell transformation is particularly well documented in gastrointestinal and blood cancers (reviewed in [10]). For instance, the PERK/eIF2α axis is shown to trigger the loss of stemness in intestinal stem cells, from which most of the colorectal cancers evolve [20,21]. Similarly, XBP1 deficiency in epithelial cells of the intestine leads to the higher incidence of colorectal cancer and colitis-associated cancer [22]. The IRE1/XBP1 pathway is also necessary for the terminal differentiation of B cells into plasma cells and is frequently upregulated in multiple myeloma (MM) [10]. Moreover, high levels of XBP1s correlate with advanced MM stages and predict poor outcome [23]. The importance of UPR signaling in cancer development is also supported by the number of cancer-associated mutations identified in the three UPR sensor-encoding genes [4]. Interestingly, the somatic mutation profiles of the UPR arms are distinct, with majority of IRE1 and ATF6 mutations reported in gastrointestinal cancers, PERK, and ATF6 in urologic and lung cancers, while ATF6 mutations were predominantly found in genital cancers [4]. Finally, elevated ER stress has been also observed in RAS-, BRAF-, MYC-, RET-, and HER2-driven tumorigenesis [24].

UPR and cancer hallmarks

Mounting evidence suggests that UPR signals support tumor progression by modulating almost all of the cancer hallmarks (as reviewed elsewhere [7,10,25,26]) (Fig. 1). Genetic ablation of IRE1/XBP1, ATF6, and PERK as well as usage of the specific inhibitors targeting the UPR arms lead to the significant reduction of

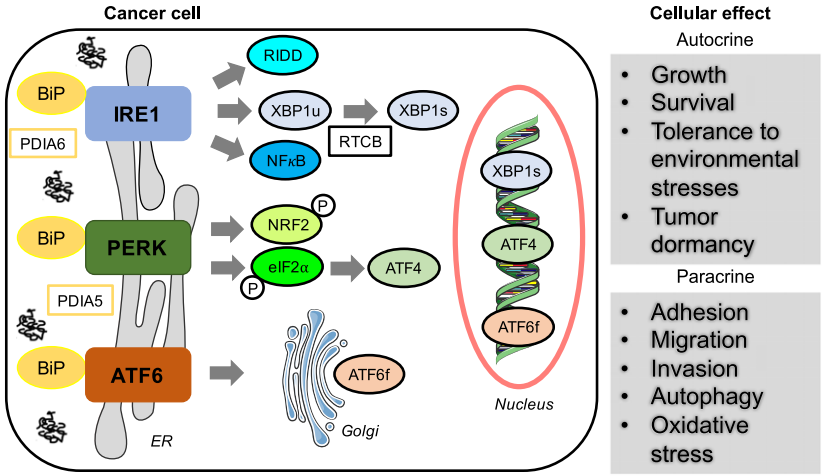


Fig. 1. Cell intrinsic effects of the UPR on cancer progression. Activation of the UPR arms—IRE1, PERK, and ATF6 activate the downstream signaling cascade driven by transcription factors XBP1s, ATF4, and ATF6f, respectively. In addition, IRE1 controls degradation of target mRNAs through RIDD and activates NFκB signaling, whereas PERK activation leads to the phosphorylation of NRF2. The integration of these outputs trigger a large number of biological effects supporting tumor progression in auto- and paracrine manner.

tumor growth both *in vitro* and *in vivo* [27–29]. PERK/eIF2α signaling is also required for tumor cells to overcome apoptosis under hypoxia, oxidative stress, or glucose deprivation [30], which involves various downstream signaling cascades, such as AKT activation, induction of glutathione synthesis, or mTOR inhibition [31–33]. In contrast, disseminated or circulating cancer cells are often exposed to the inhospitable conditions which prime them to enter a dormancy. Dormant cells are quiescent, arrested in G0/G1 cell cycle phase, and show decreased metabolic rate in order to reactivate when more favorable environmental conditions occur [34]. Interestingly, ATF6 that is constitutively activated in quiescent squamous carcinoma cells promotes cell survival in a RHEB- and mTOR-dependent manner [35]. Moreover, inhibition of ATF6 or RHEB reverts dormant tumor cell resistance to rapamycin and triggers cell death *in vivo* [35]. Similarly, both GRP78 and PERK/eIF2α are associated with increased survival and drug resistance of dormant cells [36]. Activation of the UPR also promotes cancer progression by impacting on various steps in the metastatic cascade. For instance, PERK activation is required for breast cancer cells to invade and metastasize [37]. Further, by mediating the activation of heme oxygenase 1, PERK protects fibrosarcoma cells from anoikis, thereby facilitating lung colonization [38]. PERK also promotes cancer cell migration through ATF4-dependent induction of the metastasis-associated gene LAMP3 [39]. In gastric cancer, PERK, ATF4, and ATF6 induce epithelial-to-mesenchymal transition (EMT) under severe hypoxia, which triggers TGF-β release and the concomitant activation of Smad2/3 and PI3K/AKT signaling [40]. Moreover, IRE1 contributes to the migration and adhesion of glioma cells [41], whereas XBP1 activation promotes lung metastasis in triple negative breast cancer [27]. IRE1 controls glioma cell migration partially through the degradation of SPARC mRNA and consequently inhibition of FAK and RhoA signaling [42]. In addition to its aforementioned cell intrinsic effects on tumor progression, the UPR is now becoming widely explored for its impact on tumor microenvironment, which will be further discussed in the following sections.

The UPR in the regulation of tumor microenvironment

Tumor microenvironment (TME) that comprises cancer-associated fibroblasts (CAFs), endothelial cells, and immune cells plays a key role in cancer progression. Activated CAFs fuel highly proliferating tumors with glucose, lactate, fatty acids, and amino acids and modulate signaling of adjacent cancer cells by secreting various growth factors and cytokines [43]. They can also support cancer invasion and metastasis by releasing a large number of EMT-inducing soluble factors and by remodeling the extracellular matrix [44]. Endothelial cells, which line tumor blood vessels, are educated by cancer cells to produce pro-angiogenic factors but also to promote migration, metastasis, and evade anoikis [45]. Finally, tumor-infiltrating leukocytes that include both effectors of adaptive immunity

such as T lymphocytes, dendritic cells (DCs), and B cells, as well as mediators of innate immunity, including macrophages, polymorphonuclear leukocytes, and natural killers (NK) cells, are well known to have a dual function in cancer [46]. They can eliminate cancer cells by presenting tumor-associated antigens on the MHC I and MHC II molecules, which consequently activate CD4 helper and CD8 cytotoxic T lymphocytes; however, infiltrating leukocytes can also promote tumor growth, metastasis, and chronic inflammation leading to the unfavorable patient's outcome [47]. Interestingly, a large body of evidence suggests that the UPR regulates the crosstalk between tumor and non-tumoral cells by impacting on angiogenesis, on inflammation, and on the host immune response (Fig. 2).

Transmissible UPR

The crosstalk between cancer cells and their environment depends on a variety of chemical and mechanical signals mediated by small molecules, ions, proteins, and nucleic acids. This intercellular communication occurs either through physical interactions mediated

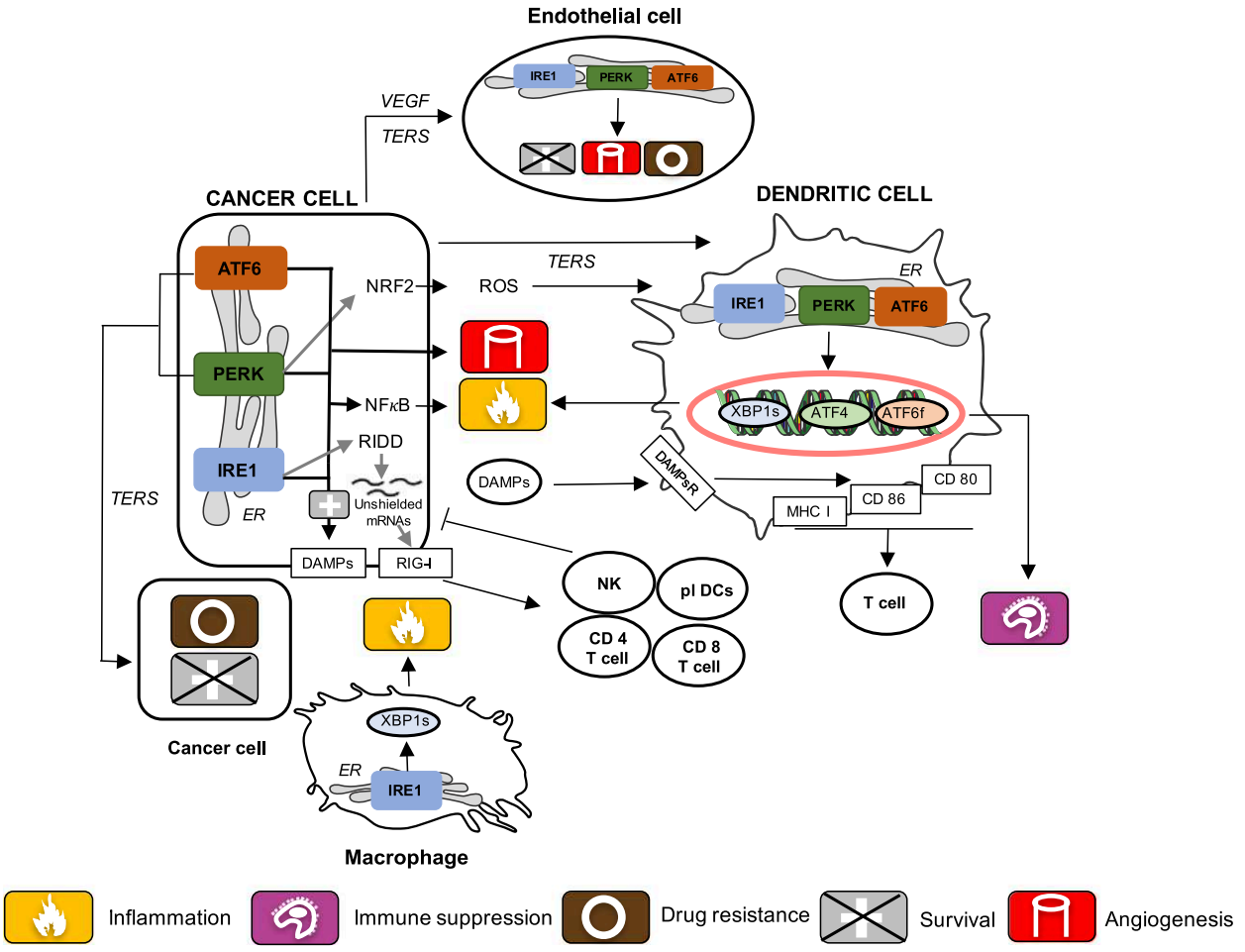


Fig. 2. UPR-mediated crosstalk between tumoral and non-tumoral cells. The UPR activation in cancer cells shapes tumor microenvironment by impacting on neighboring cancer, stromal, and immune cells. This reciprocal communication is mediated by secretion of various soluble factors including ROS, pro-angiogenetic, and pro-inflammatory molecules or by transmitting the ER stress from one cell to another (known as TERS). In that manner, TERS-imprinted juxtaposed cancer cells are resistant to apoptosis and chemotherapy. However, the non-tumoral cells, such as endothelial cells, macrophages, and dendritic cells, support cancer progression by inducing angiogenesis, inflammation, and escape from immune surveillance. However, UPR in dying cancer cells can also control the induction and/or secretion of DAMPs, which trigger the anticancer immune response through the activation of dendritic cells and consequently T cells. DAMPs, damage-associated molecular patterns; DCs, dendritic cells; NK, natural killers; pl DCs, plasmacytoid dendritic cells; ROS, reactive oxygen species; TERS, transmissible ER stress.

by gap junctions (Gap Junctional Intercellular Communication, GJIC) or remotely through the secretion of signaling molecules such as growth factors, cytokines, and exosomes. GJIC is crucial for the maintenance of tissue homeostasis by controlling growth, differentiation, and apoptosis [48,49]. In contrast, loss of direct cell–cell interaction and the lack of electrical coupling in cells are common features in many tumors. While tumor-promoting chemicals and oncogenes inhibit GJIC, antitumor chemicals and anti-oncogene drugs were associated with growth control and loss of tumorigenicity by re-gaining GJIC activity [50–58]. Moreover, the key proteins involved in GJIC, connexins are emerging as tumor suppressors [59]. For instance, the loss of connexin 32 (CX32) leads to a significant increase in liver and lung tumors in mouse models which was reversed by CX32 re-expression [60,61]. In glioma, CX43 expression was inversely correlated with tumor grade, proliferation, and migration capacities, while CX43 downregulation promotes tumor growth [62,63]. In addition to gene transcription regulation, connexin levels can be regulated by trafficking and degradation mechanisms [64]. Perturbation of protein folding in the ER has been shown to promote CX43 translocation to the cytosol and its subsequent degradation [65–69]. Moreover, CX43 mRNA and protein levels are found to be downregulated during ER stress in numerous human and mouse tumor cell lines, which reduced cell-to-extracellular matrix adhesion and increased migration [50].

Exosomes are small endosome-derived extracellular vesicles of 30- to 100-nm size secreted by a wide range of mammalian cell types, which act as mediators of cell–cell communication [70–72]. They contain a conserved set of proteins, and although they are deprived of any cellular organelles, they can still transmit variety of bioactive molecules [73], depending on the cell and tissue of origin [74,75]. Exosomes secreted by cancer cells support disease spread in both autocrine and paracrine manners by impacting on major tumor-associated pathways including cancer stemness, angiogenesis, and metastasis [76]. Exosomes can also play an important role in drug resistance mechanisms such as the expulsion of intracellular drugs and their metabolites, and neutralization of antibody-based therapies [77–82]. Due to their unique stability, selective cargo and resemblance to the cells of origin, exosomes have great potential to serve as a reservoir of cancer biomarkers for disease detection, clinical diagnosis, and selection of therapy [83–85]. Small amounts of exosomes collected from non-invasive liquid biopsies including saliva, urine, and blood [86–90] can provide multiple dynamic information from different tumors [91]. For example, exosomes released from human brain tumors were shown to contain miRNAs, different heat-shock proteins, and other tumor-promoting immunomodulatory agents that drive macrophages polarization toward pro-tumoral M2 phenotype *in vitro* [92–97]. Intriguingly, ER stress and the UPR activation enhance the exosomes' secretion. As such, tunicamycin-induced ER stress increases the multivesicular body formation in cervical cancer cells and promotes exosomes secretion in IRE1- and PERK-dependent manner, which is abrogated by PERK and IRE1 inhibition [98].

The UPR can also enhance the tumor overall fitness by being transmitted from cancer cells to the cells of TME. This transmissible ER stress (TERS) has multiple effects on the recipient cells *in vitro* and *in vivo*. For instance, TERS alters the function and cross-priming of bone marrow–derived DCs by transcriptional upregulation of different tumorigenic and immunosuppressive molecules, as well as inflammatory cytokines [99]. This phenomenon is independent of Toll-Like Receptor 2 (TLR2) or IL-6R but relies on the TLR4, which senses and potentiates TERS and conditions macrophages to mirror tumor cells [100]. Moreover, ER stress signaling pathways are triggered in mice receiving ER stress-conditioned medium that facilitates pro-tumorigenic characteristics [100,101]. In prostate cancer cells, TERS promotes survival and drug resistance to the proteasome inhibition-mediated toxicity by transmitting a unique UPR signal to the juxtaposed cancer cells. It induces Wnt signaling in an IRE1-dependent manner, while the induced enhancement of cell survival is mediated by PERK activation [102]. TERS secreted from CVB3-infected myocardiocytes also promotes the pathogenesis of viral myocarditis by augmenting the pro-inflammatory responses of cardiac infiltrating macrophages [103].

In conclusion, activation of the UPR has a broad range of targets that affects and regulates protein secretion including those involved in the intercellular communications. This important role in protein trafficking and the fact that the UPR itself can be transmitted position the UPR to influence cell–cell communication pathways and to coordinate physiological processes between cells and tissues.

UPR and angiogenesis

Angiogenesis, which is a process of remodeling existing blood vessels, involving sprouting, migration, and proliferation of endothelial cells (ECs), is mediated by several factors including PDGF, FGF, IL-8, and VEGF [104]. A growing evidence suggests that UPR plays a

key role in angiogenesis induction. It has been reported that XBP1 and ATF4 can both directly bind and transactivate VEGFA promoter in response to ER stress, an event that is even more prominent that hypoxia-driven VEGFA activation [105]. Moreover, VEGFA upregulation after oxygen or glucose deprivation is blunted in tumor cells expressing a dominant negative IRE1 as well as in IRE1-deficient mouse embryonic fibroblast (MEFs) [106]. Inhibition of IRE1 signaling decreases glioma vascular density and vessel perfusion *in vivo*, which are rescued by the expression of a transgene of IL-6 [41]. Interestingly, in diabetic bone marrow–derived progenitor cells, loss of IRE1 results in decreased angiopoietin 1 expression and disrupts angiogenesis, due to inefficient RIDD of miR-466 and miR-200 families [107]. In line with this observation, the PERK/ATF4 pathway regulates the angiogenic switch in human tumors, by increasing the expression of many proangiogenic modulators, including VEGF, FGF-2, and IL-6, with the concomitant decrease in the expression of the angiogenic inhibitors THBS1, CXCL14, and CXCL10 mRNA [108]. *In vivo*, PERK knockout in κ-Ras transformed MEFs leads to angiogenesis inhibition and reduced tumor mass compared with the wild-type counterparts [109]. More recently, it has been demonstrated that in response to acute hypoxic stress, PERK triggers the cap-independent internal ribosome entry sites-mediated translation of VEGF and FGF-2 [110]. In addition, ER stress triggered by tunicamycin, thapsigargin, or glucose deprivation also increases the expression of the pro-angiogenic factors FGF-2, IL-1α IL-6, IL-8, angiopoietin-2 and TGFβ2 [105]. Finally, at the post-translational level, ER stress induces the ER chaperone ORP150 which facilitates VEGF processing and secretion [111].

VEGF itself was shown to induce ER stress in ECs and consequently activate all three UPR branches, IRE1, ATF6, and PERK, through a PLCγ/mTORC1 pathway [112]. This VEGF-driven UPR activation is necessary for ECs survival and angiogenesis, and is mediated by AKT phosphorylation and decrease in CHOP mRNA level [112]. The UPR can be also triggered in ECs by low pH, and GRP78 has been reported to play a key role in such activation [113]. Strikingly, targeting the GRP78 in acid-stressed ECs abrogates sunitinib chemoresistance, partially through the induction of caspase 7 cleavage [113]. In renal cell carcinoma, GRP78 knockdown suppresses tumor progression and enhances the antitumor effects of anti-angiogenic therapy *in vivo* [114]. The UPR activation can be also triggered in ECs by imposing stress from adjacent cancer cells. As such, breast cancer cell-stimulated ECs upregulate the chaperone αB-crystallin (CRYAB) that acts downstream of IRE1 and ATF6 inducing VEGF expression and secretion [115]. Furthermore, by protecting endogenous VEGF from proteolytic degradation, CRYAB supports ECs proliferation and survival [115]. Taken together, activation of the UPR in cancer cells promotes angiogenesis by directly upregulating the expression of pro-angiogenic factors or transmitting the pro-angiogenic signals to the surrounding ECs.

UPR activation in immune cells

The UPR is known to control immune cell development, function, and survival both in pathological and physiological conditions. For example, highly secretory cancer cells, like B cells in multiple myeloma, produce high levels of immunoglobulins and as a consequence suffer chronic ER stress [116]. In addition, XBP1s is among the key regulators required for the activation of B-cell terminal differentiation [117]. This coincides with the fact that the upregulation of the leptin receptor upon fasting blocks acute lymphoblastic leukemia development by activating cell differentiation, which depends on the increase of the mRNA and protein levels of key transcription factors like XBP1, BLIMP1, and IRF4 [118]. In the same line, in the germinal center B-cell like (GCB) diffuse large B-cell lymphoma (DLBCL), characterized by gain-of-function mutations of EZH2, IRE1 expression levels are reduced by the binding of high amounts of H3K27me3-repressive marks to its promoter, impairing the induction of an effective ER stress response. In result, GCB-DLBCLs do not induce XBP1 splicing, contributing to accelerate tumor growth [119]. Several other studies have also suggested the importance of XBP1 during the terminal differentiation and expansion of antigen-specific CD8 T cells [120].

The IRE1/XBP1 pathway also affects conventional DCs in a tissue-specific manner. Indeed, intestinal and splenic conventional DCs survive the loss of XBP1, although with defects on their ability to cross-present dead cell-associated antigens [121,122]. This survival adaptive mechanism involves the induction of the eIF2α/ATF4/4E-BP1 pathway to avoid excessive protein loading and the IRE1/RIDD pathway to lower mRNA abundance and protein folding in the ER. Conversely, XBP1 loss affects the survival of lung and other peripheral-tissue-resident conventional DCs in a CHOP-independent manner [122]. In the context of cancer, tumor-associated DCs induce XBP1 expression in response to increased reactive oxygen species (ROS), thus modulating intracellular lipid homeostasis.

This increase in XBP1s promotes ovarian tumor growth by impairing T-cell activation [123]. In macrophages, TLR signaling inhibits the translation of ATF4 mRNA, thereby affecting the expression levels of its pro-apoptotic target CHOP. As such, macrophages can survive during the activation of the immune response [124]. However, CHOP deficiency in myeloid-derived suppressor cells shows a decreased capacity to affect T-cell responses, enhancing T-cell function and inducing an antitumor response [125]. In addition, in the MUP-uPA mouse, in which hepatocytes express high levels of urokinase plasminogen activator (uPA), and therefore undergo transient ER stress, a high fat diet induces hepatocellular carcinoma (HCC), through an ER stress-mediated mechanism that includes TNFα production by infiltrating inflammatory macrophages. Finally, both macrophage infiltration and TNF expression are inhibited by treatment with the bile acid thought to act as a chemical chaperone, tauroursodeoxycholate [126].

UPR and tumor-promoting inflammation

The UPR controls the production of inflammatory cytokines at the transcriptional and post-transcriptional level, thus having a direct impact on tumor progression. For example, XBP1s can bind to the promoter regions of IL-6 and TNFα in response to ER stress, inducing their expression in macrophages [124]. Similarly, CHOP directly regulates IL-23p19 expression in DCs [127]. IRE1, via the activation of GSK3β, induces gene expression of pro-inflammatory IL-1β, independently of its action on XBP1s signaling [128]. In addition, mTOR stimulation, also through IRE1, activates JNK and triggers IL-8 secretion in response to glutamine deprivation [129]. Interestingly, cytokines can, in turn, induce ER stress and regulate the UPR *per se*, thereby creating a feedback loop that results in the amplification of the inflammatory response. For example, in response to TNFα treatment, IKKβ phosphorylates and stabilizes XBP1s [130]. IL-10 blocks TNF-dependent translocation of cleaved ATF6 (ATF6f) to the nucleus via p38 MAPK signaling [131]. Pro-inflammatory IL-1β, IL-6, and TNFα induce the UPR and activate genes involved in the acute phase response (C-reactive protein and serum amyloid P-component) through the increased cleavage of the membrane anchored transcription factor CREBH [132]. Conversely to the great amount of studies that describe the inflammatory-dependent activation of the UPR, it has been recently described that obesity-related chronic inflammation can induce the S-nitrosylation of IRE1, thus shutting down its endoribonuclease activity without affecting its kinase domain. As a result, this modification of IRE1 contributes to metabolic and inflammatory stress, compromising the adaptive UPR response through the decrease of XBP1s followed by an increase in JNK levels [133].

Interestingly, aberrant lipid composition of the ER membrane (also known as lipid bilayer stress) can activate IRE1. It has been suggested that IRE1, due to its localization and mechanistic properties, can sense and signal lipid changes in the ER membrane independently of misfolded protein accumulation in the ER lumen. This occurs by inducing IRE1 oligomerization and signaling, hence recognizing IRE1 as a molecular link between protein and lipid homeostasis [134,135]. IRE1 activation also allows the crosstalk between the ER and the mitochondria through the ROS-dependent activation of the NLRP3 inflammasome. This results in the activation of caspase-2, the cleavage of the pro-apoptotic factor BID, and the release of mitochondrial contents [136]. These studies bring new membrane-based perspectives to the role of lipids in the ER stress-related pathophysiological conditions.

The UPR crosstalks with numerous other signaling pathways to regulate tumor–host interactions. The three branches of the UPR have been shown to induce the pro-inflammatory NFκB pathway. First, IRE1 interacts with TRAF2, recruiting IKK and inducing the phosphorylation and degradation of IκB, which allows NFκB to translocate to the nucleus. Second, IκB has a shorter half-life than NFκB and for this reason, changes in protein translation under ER stress stimuli that activate the PERK pathway, induce the NFκB pathway by decreasing the IκB:NFκB ratio. Finally, ATF6 can induce NFκB through the phosphorylation of AKT [120].

UPR and antitumor immune response

There is a growing evidence that the UPR can also control the antitumor immune response by acting as an innate immune machinery. The UPR can regulate the release of damage-associated molecular patterns (DAMPs), which can act as 'eat me' signals, 'find me' signals, or chemoattractants. In summary, DAMPs are intracellular molecules that are hidden from the immune system's recognition under normal conditions. However, upon cellular stress or death, cells can induce an immunogenic response by the pre-apoptotic expression of DAMPs on the cell surface [e.g. calreticulin (CALR) and heat-shock proteins (HSPs)] or by releasing or secreting them (e.g. ATP and HMGB1) [120,137]. This type of cell death is known as

immunogenic cell death (ICD). Interestingly, ICD has to be preceded by the ER stress in order to induce CALR and HSPs surface exposure. In the case of CALR exposure, it occurs through the activation of the PERK/eIF2α pathway [138]; however, the exact respective contribution of PERK activation and eIF2α phosphorylation need to be further explored. Hand, ATP release depends on premortem autophagy and the secretion of HMGB1 on secondary necrosis [137].

These DAMPs are recognized by specific receptors: CALR binds to CD91, ATP binds to purinergic receptors (P2Y2 or P2X7), and HMGB1 binds to TLR4, respectively [137]. These receptors are found on DCs and promote engulfment of dying cells, attraction of DCs into the tumor bed, production of IL-1β, and tumor antigen presentation. CALR is a highly conserved calcium-binding ER lectin that has important functions in the immune response. For example, CALR chaperones MHC class I molecules, thus regulating antigen presentation hence affecting recognition by CD8 T cells [139]. It is also associated with the increased expression of CD86, CD80, and MHC II in the cell surface of DCs, leading to an efficient anticancer CD8 T-cell response [120]. Furthermore, CALR exposure at the cell surface plays an important role in the immunosurveillance mechanism induced by cells that have increased ploidy [140]. Even though ER stress induces CALR exposure to the cell surface during ICD, the mechanism by which this phenomenon happens remains elusive.

HMGB1 secretion during cell death can activate the UPR in DCs by increasing GRP78 expression and XBP1 splicing [120]. XBP1 silencing leads to the downregulation of CD86 and CD80 cell surface activation markers and MHC class II expression. These events result in the decrease of T-cell proliferation and differentiation affecting the activation of T cells in *ex vivo* co-cultures [120]. In more recent studies, increased expression of HMGB1, HMGN1, XBP1, and peIF2α is correlated with a high amount of tumor-infiltrating lymphocytes in triple negative breast cancer patients [141]. Besides DAMPs, there are also 'don't eat me' signals that will help cancer cells avoid the immune system's recognition. ER stress regulated proteins also control these signals. For example, GRP78 inhibition in BALB/c and athymic tumor-bearing mice increases monocyte chemoattractant protein-1 (MCP-1) serum levels and regulates CD47, a glycoprotein of the immunoglobulin superfamily critical in self-recognition. Normal tissue increases the CD47 'don't eat me' signal in response to GRP78 inhibition, while the tumoral tissue decreases its expression. In this way, GRP78 inhibition stimulates macrophage infiltration and reduction of estrogen receptor–positive breast cancers [142].

The similarities between the antigen-specific immune response triggered by ICD and those induced by pathogen infection have led scientists to look into these pathways in order to try to apply this knowledge in cancer research. This is the case of TLRs, which are pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns. Activation of TLRs and the IRE1/XBP1 pathway are interconnected and result in the induction of the innate immune surveillance in response to pathogen infection. In macrophages, TLR activation induces a ROS-dependent-specific activation of the IRE1α/XBP1 pathway, but not of the other arms of the UPR. Then, XBP1 induces IL-6 and IFN-β cytokine production [124]. This kind of response is not restricted to TLRs, as there is a clear link between the UPR and retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs). RLRs are RNA helicases that sense pathogenic RNA and initiate antiviral immunity. Recent studies have linked IRE1 with the RIG-I pathway upon pathogen infection [143] and pathological conditions [144]. Upon the activation of IRE1 RNase activity, the cleavage of endogenous RNA through RIDD may produce fragments that resemble those of pathogens as they lack 5′-caps or 3′-polyA-tails that mark mRNA as 'self'. These fragments, in turn, activate RIG-I that induces an innate immune response.

In the context of cancer, endogenous RNAs that are not shielded by RNA binding proteins have already been shown to act as DAMPs for PRRs. In primary human breast cancers, activated stromal cells present unshielded RNA in exosomes in order to propagate antiviral signaling to the TME. This unshielded RNA in stromal exosomes results in an inflammatory response when transferred to immune cells and in tumor growth and invasion when transferred to breast cancer cells [145]. In immunocompetent mice, RIG-I activation induces the secretion of extracellular vesicles by melanoma cells that act as immune activating agents favoring the anticancer immune response [146]. Administration of a BCL-2 siRNA activates RIG-I efficiently and leads to tumor growth inhibition through an antitumor immune response. This antitumor response involves myeloid and plasmacytoid DC activation, NK cells, CD4 and CD8 T cells and is associated with the secretion of type I cytokines (IFN-α, IL-12p40, and IFN-γ) [147]. Furthermore, RIG-I has been proposed as a tumor suppressor in HCC as RIG-I deficiency promotes HCC carcinogenesis [148]. Other studies in highly immunodeficient mice suggest that RIG-I can inhibit

tumor growth by inducing apoptosis through the regulation of BH3-only proteins [149]. Additionally, pancreatic cancer cells treated with RIG-I–like helicase ligands die through ICD. This ICD occurs through the translocation of CALR to the cell surface followed by the release of HMGB1 that activates DCs and cytotoxic CD8 T cells [150]. One could speculate that stimuli activating the IRE1/RIDD pathway in tumor cells could potentially activate RIG-I, inducing ICD and an anticancer immune response. Altogether, these studies highlight the importance of studying the regulation of the UPR in the context of cancer in order to understand immunogenicity and to improve the antitumor immune responses and therapies.

Impact of UPR activation in tumor-surrounding parenchyma

Tumors develop in particular tissular environments that are composed by a multiple of non-tumoral cell types. Next to ECs and immune cells (presented above), other stromal/parenchymal cells as such stellate cells, epithelial cells, fibroblasts or astrocytes, and neurons could also be affected by UPR downstream signals provided by tumor cells. Little if any of such interactions is up-to-now reported in the context of neoplasia, and only few indirect evidences are described so far. For instance, pancreatic stellate cells are key stromal cells in pancreatic cancer for secreting extracellular matrix proteins and inflammatory mediators. Under metabolic stress, the PERK/CHOP branch of the UPR is activated in pancreatic stellate cells, thus protecting them from apoptosis [151]. Furthermore, under these conditions, stellate cell fibrogenic activity is reduced and the profile of secreted cytokines is modulated (i.e. reduction of IL-6 expression and increase of the immune modulator IL-4), thereby contributing to the modulation of TME. Several UPR-induced genes have clear impact on the stromal cells surrounding tumors. For instance, Serpin B2 expressed by the cancer-associated fibroblasts limits metastasis in pancreatic ductal adenocarcinoma due to its collagen remodeling capacity [152]. ATF3, a downstream effector of the PERK/ATF4 pathway, is a key regulator of tumor-associated stromal cell reprogramming leading to increase in their proliferation ability, which in turn supports tumor growth [153]. The impact of the UPR on stromal cells is well documented in other pathologic situations such as neurogenerative diseases [154]. Indeed, instead of improving protein quality control and protein folding, prolonged ER stress leads to neuronal cell apoptosis, synaptic dysfunction, and axonal degeneration. One could speculate that brain tumor transmissible UPR would affect neighboring brain resident cells such as oligodendrocytes, astrocytes, and neurons leading to neuronal dysfunctions and tumor cell bedding. Further studies are required to investigate such stroma/tumor cell communications through UPR activation.

Targeting UPR as anticancer approach

As exemplify above, the UPR has a broad impact on tumor-associated processes such as sustained growth, resistance to apoptosis, metastasis, inflammation, or escape from immune recognition, which creates a rationale for targeting ER stress pathways as a potential anticancer approach. This can be achieved either by exploiting the pro-death UPR signaling to effectively kill cancer cells or to impede UPR-mediated adaptive responses which help tumor cells propagate in harsh TME conditions and resist the treatment. As such, ER stress- triggered apoptosis has been observed in various cancer models both *in vitro* and *in vivo*. For instance, in glioblastoma, a large number of small molecules including FDA-approved drugs nelfinavir, quinine, and celecoxib have been reported to induce cell death by perturbing ER proteostasis, which is mainly mediated by the upregulation of GRP78 and/or CHOP mRNA or protein levels [29]. Further, many natural and chemical agents are shown to promote cell death by generating ROS and consequently triggering ER stress in lung, breast, liver, or colon cancer [155]. As discussed above, the UPR has a very important role in ICD induction and constitutes a promising target for the development of novel anticancer strategies. Remarkably, patients can only benefit of checkpoint blockade immunotherapies if tumors are infiltrated by tumor-infiltrating lymphocytes (TILs) previous to the treatment. Importantly, tumors without TILs can be sensitized to checkpoint blockade immunotherapies when combined with ICD-inducing drugs [123]. In this sense, the co-administration of chemotherapies that do not induce ICD with immunogenic chemotherapies capable of inducing the UPR should be considered. Many of the ICD inducers are intensively used in the clinical practice and are divided into the type I and type II ICD inducers [156,157]. On one hand, type I inducers, such as bortezomib, anthracyclines, and oxaliplatin, trigger apoptosis via non-ER targets (e.g. through the DNA damage or proteasomal inhibition) with the parallel 'off-target' impact on the ER stress signaling [157]. On the other hand, type II ICD inducers (involving hypericin-photodynamic therapy and oncolytic viruses) drive apoptosis through the selective activation of ROS-mediated ER stress [157].

Interestingly, it has been recently demonstrated that cells resistant to ER stress and chemotherapy acquire a multidrug-resistant phenotype through the activation of the PERK/NRF2/MRP1 signaling axis. Targeting this axis restores chemosensitivity in resistant cancer cells and diminishes tumor growth *in vivo* [158].

In the past decade, various inhibitors targeting each of the UPR arm have been developed and have been shown to yield a promising antitumor response (Fig. 3). As such, four compounds are reported to modulate ATF6 signaling: 16F16 (a PDI inhibitor necessary for ATF6 activation), caepins, and two non-toxic ATF6 activators: compounds 147 and 263 [29]. Interestingly, impairing ATF6 signaling with 16F16 restores imatinib sensitivity in imatinib-resistant leukemia K562 cells [159]. More recently, it has been showed that melatonin blocks the ATF6 signaling in HCC leading to a decrease in COX-2 expression and consequently promoting cell apoptosis under tunicamycin-induced ER stress [160]. The IRE1 modulators developed so far include both RNase inhibitors such as 4μ8C, ManKindCorp (MKC) analogs, 3-methoxy-6-bromosalicylaldehyde, and STF-083010, as well as agents targeting kinase domain—KIRAs and ATP kinase inhibitor compound 3 [29]. Those inhibitors are shown to kill cancer cells or sensitize them to common chemo- or radiotherapies. For instance, STF-083010 significantly inhibits the growth of human multiple myeloma xenografts [161]. Moreover, it restores tamoxifen sensitivity in resistant breast cancer cells, while when administered synergistically with tamoxifen, it suppresses breast tumor progression *in vivo* [162]. Similarly, MKC-3946 decreases multiple myeloma growth and shows therapeutic activity in the combination with the proteasome inhibitor bortezomib [163]. Finally, the KIRA6 inhibitor and the optimized KIRA, KIRA8 which is a mono-selective IRE1 inhibitor with a single digit nanomolar potency, block IRE1 *in vivo* and promote cell survival under ER stress in several mouse models [118,164]. Regarding PERK inhibitors, GSK2656157, ISRIB, salubrinal, guanabenz, and sephin 1/IFB-088 are shown to modify PERK phosphorylation or its downstream signaling by targeting the eIF2α complexes [29]. GSK2606414 and the related drug GSK2656157 impact on cancer progression by decreasing tumor growth and reducing tumor-associated angiogenesis, respectively [165,166]. Moreover, in an orthotopic model of pancreatic ductal adenocarcinoma, ISRIB enhances the gemcitabine chemosensitivity by suppressing the integrated stress response and its downstream anti-apoptotic pathways [167]. In line, salubrinal, an inhibitor of growth arrest and DNA damage 34 (GADD34), potentiates the cytotoxic effect of doxorubicin in doxorubicin-resistant breast cancer cells *in vitro* [168]. Thus, molecules that generate irremediable ER stress in tumor cells or specifically target the UPR branches represent interesting therapeutic options alone or in combination with other commonly used drugs.

Concluding remarks

The UPR signaling has a broad impact on cancer biology. It not only provides tumor cells with the selective advantages to survive and propagate in harsh environmental conditions but also educates the surrounding non-tumoral cells to even further promote cancer progression. As discussed herein, signals emerging from the ER impact on the tumor secretome, which in turn supports new vessels formation, inflammation, or immune suppression. Interestingly, a growing evidence suggests the UPR involvement in the regulation of antitumor host response. Nevertheless, we still need to uncover what triggers these opposite outputs. Apparently, the

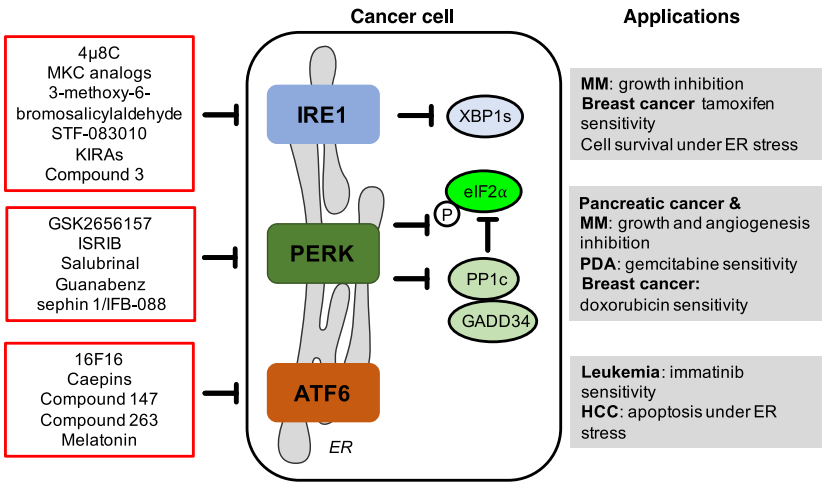


Fig. 3. Anticancer effects of the UPR-targeting drugs. Many molecules specifically targeting each of the UPR branches show potential anticancer activities either by inhibiting tumor growth or restoring chemosensitivity in drug-resistant cells.

difference lays in a combination of the type of UPR-inducing stimulus and which pathways are engaged in response to it. It is tempting to think that it all comes down to a fine-tuning of the different UPR proteins downstream of the master sensors. For example, in the case of IRE1, which has an interesting dual role in both cell death and immunosurveillance, several pathways ramify downstream of its activation. Is the outcome the same if we activate more the XBP1 downstream pathway than the RIDD pathway and *vice versa*? Further studies are needed in order to complete the puzzle that is the UPR in the TME and its control of cell death and the immune system response. For these reasons, modulating ER stress in tumor cells and the TME represents an additional level of therapeutic intervention.

Acknowledgements

This work was funded by grants from Institut National du Cancer (INCa; PLBIO: 2017, PLBIO: 2015-111, INCA_7981), and Ligue Contre le Cancer, EU H2020 MSCA ITN-675448 (TRAINERS) and MSCA RISE-734749 (INSPIRED) to EC; Fondation ARC pour la Recherche sur le Cancer and the Agence Nationale de la Recherche (LABEX SIGNALIFE ANR-11-LABX-0028-01) to JER; Juvenile Diabetes Research Foundation (JDRF 3-PDF-2015-80-A-N) to AI.

Author contributions

JPB, CR-P, and J-ER wrote the section on the interaction between tumor cells and the immune system; AI wrote the section on UPR transmissibility. JO, TA, and EC wrote the rest of the manuscript.

References

1 Dejeans N, Manie S, Hetz C, Bard F, Hupp T, Agostinis P, Samali A & Chevet E (2014) Addicted to secrete - novel concepts and targets in cancer therapy. *Trends Mol Med* **20**, 242–250.
2 Hetz C, Chevet E & Oakes SA (2015) Proteostasis control by the unfolded protein response. *Nat Cell Biol* **17**, 829–838.
3 Ron D & Walter P (2007) Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* **8**, 519–529.
4 Chevet E, Hetz C & Samali A (2015) Endoplasmic reticulum stress-activated cell reprogramming in oncogenesis. *Cancer Discov* **5**, 586–597.
5 Gardner BM & Walter P (2011) Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. *Science* **333**, 1891–1894.
6 Karagoz GE, Acosta-Alvear D, Nguyen HT, Lee CP, Chu F & Walter P (2017) An unfolded protein-induced conformational switch activates mammalian IRE1. *Elife* **6**, pii: e30700.
7 Urra H, Dufey E, Avril T, Chevet E & Hetz C (2016) Endoplasmic reticulum stress and the hallmarks of cancer. *Trends Cancer* **2**, 252–262.
8 Le Reste PJ, Avril T, Quillien V, Morandi X & Chevet E (2016) Signaling the unfolded protein response in primary brain cancers. *Brain Res* **1642**, 59–69.
9 Hetz C (2012) The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat Rev Mol Cell Biol* **13**, 89–102.
10 Dejeans N, Barroso K, Fernandez-Zapico ME, Samali A & Chevet E (2015) Novel roles of the unfolded protein response in the control of tumor development and aggressiveness. *Semin Cancer Biol* **33**, 67–73.
11 Walter P & Ron D (2011) The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **334**, 1081–1086.
12 Hetz C, Martinon F, Rodriguez D & Glimcher LH (2011) The unfolded protein response: integrating stress signals through the stress sensor IRE1alpha. *Physiol Rev* **91**, 1219–1243.
13 Maurel M, Chevet E, Tavernier J & Gerlo S (2014) Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem Sci* **39**, 245–254.
14 Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP & Ron D (2000) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* **287**, 664–666.
15 Maurel M, McGrath EP, Mnich K, Healy S, Chevet E & Samali A (2015) Controlling the unfolded protein response-mediated life and death decisions in cancer. *Semin Cancer Biol* **33**, 57–66.
16 Sato M, Yao VJ, Arap W & Pasqualini R (2010) GRP78 signaling hub a receptor for targeted tumor therapy. *Adv Genet* **69**, 97–114.
17 Ren P, Chen C, Yue J, Zhang J & Yu Z (2017) High expression of glucose-regulated protein 78 (GRP78) is associated with metastasis and poor prognosis in patients with esophageal squamous cell carcinoma. *Onco Targets Ther* **10**, 617–625.
18 Yang L, Yang S, Liu J, Wang X, Ji J, Cao Y, Lu K, Wang J & Gao Y (2014) Expression of GRP78 predicts taxane-based therapeutic resistance and recurrence of human gastric cancer. *Exp Mol Pathol* **96**, 235–241.
19 Zheng HC, Takahashi H, Li XH, Hara T, Masuda S, Guan YF & Takano Y (2008) Overexpression of GRP78 and GRP94 are markers for aggressive behavior and poor prognosis in gastric carcinomas. *Hum Pathol* **39**, 1042–1049.

20 Heijmans J, van Lidth de Jeude JF, Koo BK, Rosekrans SL, Wielenga MC, van de Wetering M, Ferrante M, Lee AS, Onderwater JJ, Paton JC *et al.* (2013) ER stress causes rapid loss of intestinal epithelial stemness through activation of the unfolded protein response. *Cell Rep* **3**, 1128–1139.
21 Vermeulen L & Snippert HJ (2014) Stem cell dynamics in homeostasis and cancer of the intestine. *Nat Rev Cancer* **14**, 468–480.
22 Niederreiter L, Fritz TM, Adolph TE, Krismer AM, Offner FA, Tschurtschenthaler M, Flak MB, Hosomi S, Tomczak MF, Kaneider NC *et al.* (2013) ER stress transcription factor Xbp1 suppresses intestinal tumorigenesis and directs intestinal stem cells. *J Exp Med* **210**, 2041–2056.
23 Vincenz L, Jager R, O'Dwyer M & Samali A (2013) Endoplasmic reticulum stress and the unfolded protein response: targeting the Achilles heel of multiple myeloma. *Mol Cancer Ther* **12**, 831–843.
24 Tameire F, Verginadis II & Koumenis C (2015) Cell intrinsic and extrinsic activators of the unfolded protein response in cancer: mechanisms and targets for therapy. *Semin Cancer Biol* **33**, 3–15.
25 Dufey E, Urra H & Hetz C (2015) ER proteostasis addiction in cancer biology: novel concepts. *Semin Cancer Biol* **33**, 40–47.
26 Oakes SA (2017) Endoplasmic reticulum proteostasis: a key checkpoint in cancer. *Am J Physiol Cell Physiol* **312**, C93–C102.
27 Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, Lim E, Tam WL, Ni M, Chen Y *et al.* (2014) XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. *Nature* **508**, 103–107.
28 Hou X, Liu Y, Liu H, Chen X, Liu M, Che H, Guo F, Wang C, Zhang D, Wu J *et al.* (2015) PERK silence inhibits glioma cell growth under low glucose stress by blockage of p-AKT and subsequent HK2's mitochondria translocation. *Sci Rep* **5**, 9065.
29 Obacz J, Avril T, Le Reste PJ, Urra H, Quillien V, Hetz C & Chevet E (2017) Endoplasmic reticulum proteostasis in glioblastoma-From molecular mechanisms to therapeutic perspectives. *Sci Signal* **10**, pii: eaal2323.
30 Bu Y & Diehl JA (2016) PERK integrates oncogenic signaling and cell survival during cancer development. *J Cell Physiol* **231**, 2088–2096.
31 Blaustein M, Perez-Munizaga D, Sanchez MA, Urrutia C, Grande A, Risso G, Srebrow A, Alfaro J & Colman-Lerner A (2013) Modulation of the Akt pathway reveals a novel link with PERK/eIF2alpha, which is relevant during hypoxia. *PLoS One* **8**, e69668.
32 Rouschop KM, Dubois LJ, Keulers TG, van den Beucken T, Lambin P, Bussink J, van der Kogel AJ, Koritzinsky M & Wouters BG (2013) PERK/eIF2alpha signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. *Proc Natl Acad Sci USA* **110**, 4622–4627.
33 Freis P, Bollard J, Lebeau J, Massoma P, Fauvre J, Vercherat C, Walter T, Manie S, Roche C, Scoazec JY *et al.* (2017) mTOR inhibitors activate PERK signaling and favor viability of gastrointestinal neuroendocrine cell lines. *Oncotarget* **8**, 20974–20987.
34 Sosa MS, Bragado P & Aguirre-Ghiso JA (2014) Mechanisms of disseminated cancer cell dormancy: an awakening field. *Nat Rev Cancer* **14**, 611–622.
35 Schewe DM & Aguirre-Ghiso JA (2008) ATF6alpha-Rheb-mTOR signaling promotes survival of dormant tumor cells in vivo. *Proc Natl Acad Sci USA* **105**, 10519–10524.
36 Ranganathan AC, Zhang L, Adam AP & Aguirre-Ghiso JA (2006) Functional coupling of p38-induced up-regulation of BiP and activation of RNA-dependent protein kinase-like endoplasmic reticulum kinase to drug resistance of dormant carcinoma cells. *Cancer Res* **66**, 1702–1711.
37 Feng YX, Sokol ES, Del Vecchio CA, Sanduja S, Claessen JH, Proia TA, Jin DX, Reinhardt F, Ploegh HL, Wang Q *et al.* (2014) Epithelial-to-mesenchymal transition activates PERK-eIF2alpha and sensitizes cells to endoplasmic reticulum stress. *Cancer Discov* **4**, 702–715.
38 Dey S, Sayers CM, Verginadis II, Lehman SL, Cheng Y, Cerniglia GJ, Tuttle SW, Feldman MD, Zhang PJ, Fuchs SY *et al.* (2015) ATF4-dependent induction of heme oxygenase 1 prevents anoikis and promotes metastasis. *J Clin Invest* **125**, 2592–2608.
39 Mujcic H, Nagelkerke A, Rouschop KM, Chung S, Chaudary N, Span PN, Clarke B, Milosevic M, Sykes J, Hill RP *et al.* (2013) Hypoxic activation of the PERK/eIF2alpha arm of the unfolded protein response promotes metastasis through induction of LAMP3. *Clin Cancer Res* **19**, 6126–6137.
40 Shen X, Xue Y, Si Y, Wang Q, Wang Z, Yuan J & Zhang X (2015) The unfolded protein response potentiates epithelial-to-mesenchymal transition (EMT) of gastric cancer cells under severe hypoxic conditions. *Med Oncol* **32**, 447.
41 Auf G, Jabouille A, Guerit S, Pineau R, Delugin M, Bouchecareilh M, Magnin N, Favereaux A, Maitre M, Gaiser T *et al.* (2010) Inositol-requiring enzyme 1alpha is a key regulator of angiogenesis and invasion in malignant glioma. *Proc Natl Acad Sci USA* **107**, 15553–15558.
42 Dejeans N, Pluquet O, Lhomond S, Grise F, Bouchecareilh M, Juin A, Meynard-Cadars M, Bidaud-Meynard A, Gentil C, Moreau V *et al.* (2012) Autocrine control of glioma cells adhesion

and migration through IRE1alpha-mediated cleavage of SPARC mRNA. *J Cell Sci* **125**, 4278–4287.
43 Lyssiotis CA & Kimmelman AC (2017) Metabolic interactions in the tumor microenvironment. *Trends Cell Biol* **27**, 863–875.
44 Mezawa Y & Orimo A (2016) The roles of tumor- and metastasis-promoting carcinoma-associated fibroblasts in human carcinomas. *Cell Tissue Res* **365**, 675–689.
45 Maishi N & Hida K (2017) Tumor endothelial cells accelerate tumor metastasis. *Cancer Sci* **108**, 1921–1926.
46 Whiteside TL (2008) The tumor microenvironment and its role in promoting tumor growth. *Oncogene* **27**, 5904–5912.
47 Fridman WH, Zitvogel L, Sautes-Fridman C & Kroemer G (2017) The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol* **14**, 717–734.
48 Goldmann WH (2002) Mechanical aspects of cell shape regulation and signaling. *Cell Biol Int* **26**, 313–317.
49 Jongsma HJ & Wilders R (2000) Gap junctions in cardiovascular disease. *Circ Res* **86**, 1193–1197.
50 Huang T, Wan Y, Zhu Y, Fang X, Hiramatsu N, Hayakawa K, Paton AW, Paton JC, Kitamura M & Yao J (2009) Downregulation of gap junction expression and function by endoplasmic reticulum stress. *J Cell Biochem* **107**, 973–983.
51 Yamasaki H, Mesnil M, Omori Y, Mironov N & Krutovskikh V (1995) Intercellular communication and carcinogenesis. *Mutat Res* **333**, 181–188.
52 Yang S, Wen Q, Liu Y, Zhang C, Wang M, Chen G, Gong Y, Zhong J, Chen X, Stucky A *et al.* (2015) Increased expression of CX43 on stromal cells promotes leukemia apoptosis. *Oncotarget* **6**, 44323–44331.
53 Yamasaki H (1990) Gap junctional intercellular communication and carcinogenesis. *Carcinogenesis* **11**, 1051–1058.
54 Kumar NM & Gilula NB (1996) The gap junction communication channel. *Cell* **84**, 381–388.
55 Aasen T, Mesnil M, Naus CC, Lampe PD & Laird DW (2017) Gap junctions and cancer: communicating for 50 years. *Nat Rev Cancer* **17**, 74.
56 Aasen T, Mesnil M, Naus CC, Lampe PD & Laird DW (2016) Gap junctions and cancer: communicating for 50 years. *Nat Rev Cancer* **16**, 775–788.
57 Trosko JE & Chang CC (2001) Mechanism of up-regulated gap junctional intercellular communication during chemoprevention and chemotherapy of cancer. *Mutat Res* **480–481**, 219–229.
58 Huang RP, Hossain MZ, Huang R, Gano J, Fan Y & Boynton AL (2001) Connexin 43 (cx43) enhances chemotherapy-induced apoptosis in human glioblastoma cells. *Int J Cancer* **92**, 130–138.
59 Raza A, Ghoshal A, Chockalingam S & Ghosh SS (2017) Connexin-43 enhances tumor suppressing activity of artesunate via gap junction-dependent as well as independent pathways in human breast cancer cells. *Sci Rep* **7**, 7580.
60 King TJ & Lampe PD (2004) The gap junction protein connexin32 is a mouse lung tumor suppressor. *Cancer Res* **64**, 7191–7196.
61 Koffler L, Roshong S, Kyu Park I, Cesen-Cummings K, Thompson DC, Dwyer-Nield LD, Rice P, Mamay C, Malkinson AM & Ruch RJ (2000) Growth inhibition in G(1) and altered expression of cyclin D1 and p27(kip-1)after forced connexin expression in lung and liver carcinoma cells. *J Cell Biochem* **79**, 347–354.
62 Leithe E, Mesnil M & Aasen T (2017) The connexin 43 C-terminus: a tail of many tales. *Biochim Biophys Acta* **1860**, 48–64.
63 Ye XY, Jiang QH, Hong T, Zhang ZY, Yang RJ, Huang JQ, Hu K & Peng YP (2015) Altered expression of connexin43 and phosphorylation connexin43 in glioma tumors. *Int J Clin Exp Pathol* **8**, 4296–4306.
64 Su V & Lau AF (2014) Connexins: mechanisms regulating protein levels and intercellular communication. *FEBS Lett* **588**, 1212–1220.
65 Musil LS, Le AC, VanSlyke JK & Roberts LM (2000) Regulation of connexin degradation as a mechanism to increase gap junction assembly and function. *J Biol Chem* **275**, 25207–25215.
66 VanSlyke JK, Deschenes SM & Musil LS (2000) Intracellular transport, assembly, and degradation of wild-type and disease-linked mutant gap junction proteins. *Mol Biol Cell* **11**, 1933–1946.
67 VanSlyke JK & Musil LS (2002) Dislocation and degradation from the ER are regulated by cytosolic stress. *J Cell Biol* **157**, 381–394.
68 VanSlyke JK & Musil LS (2003) Degradation of connexins from the plasma membrane is regulated by inhibitors of protein synthesis. *Cell Commun Adhes* **10**, 329–333.
69 VanSlyke JK & Musil LS (2005) Cytosolic stress reduces degradation of connexin43 internalized from the cell surface and enhances gap junction formation and function. *Mol Biol Cell* **16**, 5247–5257.
70 Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W & Geuze HJ (2000) Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. *J Cell Sci* **113** (Pt 19), 3365–3374.
71 Schneider A & Simons M (2013) Exosomes: vesicular carriers for intercellular communication in neurodegenerative disorders. *Cell Tissue Res* **352**, 33–47.
72 Simons M & Raposo G (2009) Exosomes–vesicular carriers for intercellular communication. *Curr Opin Cell Biol* **21**, 575–581.

73 Batista BS, Eng WS, Pilobello KT, Hendricks-Munoz KD & Mahal LK (2011) Identification of a conserved glycan signature for microvesicles. *J Proteome Res* **10**, 4624–4633.

74 Zhang J, Li S, Li L, Li M, Guo C, Yao J & Mi S (2015) Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics Proteomics Bioinformatics* **13**, 17–24.

75 Gibbings DJ, Ciaudo C, Erhardt M & Voinnet O (2009) Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nat Cell Biol* **11**, 1143–1149.

76 Ramteke A, Ting H, Agarwal C, Mateen S, Somasagara R, Hussain A, Graner M, Frederick B, Agarwal R & Deep G (2015) Exosomes secreted under hypoxia enhance invasiveness and stemness of prostate cancer cells by targeting adherens junction molecules. *Mol Carcinog* **54**, 554–565.

77 Chen WX, Liu XM, Lv MM, Chen L, Zhao JH, Zhong SL, Ji MH, Hu Q, Luo Z, Wu JZ *et al.* (2014) Exosomes from drug-resistant breast cancer cells transmit chemoresistance by a horizontal transfer of microRNAs. *PLoS One* **9**, e95240.

78 Ciravolo V, Huber V, Ghedini GC, Venturelli E, Bianchi F, Campiglio M, Morelli D, Villa A, Della Mina P, Menard S *et al.* (2012) Potential role of HER2-overexpressing exosomes in countering trastuzumab-based therapy. *J Cell Physiol* **227**, 658–667.

79 Wei Y, Lai X, Yu S, Chen S, Ma Y, Zhang Y, Li H, Zhu X, Yao L & Zhang J (2014) Exosomal miR-221/222 enhances tamoxifen resistance in recipient ER-positive breast cancer cells. *Breast Cancer Res Treat* **147**, 423–431.

80 Safaei R, Larson BJ, Cheng TC, Gibson MA, Otani S, Naerdemann W & Howell SB (2005) Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells. *Mol Cancer Ther* **4**, 1595–1604.

81 Chen VY, Posada MM, Blazer LL, Zhao T & Rosania GR (2006) The role of the VPS4A-exosome pathway in the intrinsic egress route of a DNA-binding anticancer drug. *Pharm Res* **23**, 1687–1695.

82 Epple LM, Dodd RD, Merz AL, Dechkovskaia AM, Herring M, Winston BA, Lencioni AM, Russell RL, Madsen H, Nega M *et al.* (2013) Induction of the unfolded protein response drives enhanced metabolism and chemoresistance in glioma cells. *PLoS One* **8**, e73267.

83 Panigrahi GK & Deep G (2017) Exosomes-based biomarker discovery for diagnosis and prognosis of prostate cancer. *Front Biosci (Landmark Ed)* **22**, 1682–1696.

84 Properzi F, Logozzi M & Fais S (2013) Exosomes: the future of biomarkers in medicine. *Biomark Med* **7**, 769–778.

85 Nedaeinia R, Manian M, Jazayeri MH, Ranjbar M, Salehi R, Sharifi M, Mohaghegh F, Goli M, Jahednia SH, Avan A *et al.* (2017) Circulating exosomes and exosomal microRNAs as biomarkers in gastrointestinal cancer. *Cancer Gene Ther* **24**, 48–56.

86 Zlotogorski-Hurvitz A, Dayan D, Chaushu G, Korvala J, Salo T, Sormunen R & Vered M (2015) Human saliva-derived exosomes: comparing methods of isolation. *J Histochem Cytochem* **63**, 181–189.

87 Palanisamy V, Sharma S, Deshpande A, Zhou H, Gimzewski J & Wong DT (2010) Nanostructural and transcriptomic analyses of human saliva derived exosomes. *PLoS One* **5**, e8577.

88 Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G & Bonnerot C (2005) Exosomal-like vesicles are present in human blood plasma. *Int Immunol* **17**, 879–887.

89 Almqvist N, Lonnqvist A, Hultkrantz S, Rask C & Telemo E (2008) Serum-derived exosomes from antigen-fed mice prevent allergic sensitization in a model of allergic asthma. *Immunology* **125**, 21–27.

90 Chen CY, Hogan MC & Ward CJ (2013) Purification of exosome-like vesicles from urine. *Methods Enzymol* **524**, 225–241.

91 Peterson MF, Otoc N, Sethi JK, Gupta A & Antes TJ (2015) Integrated systems for exosome investigation. *Methods* **87**, 31–45.

92 Harshyne LA, Nasca BJ, Kenyon LC, Andrews DW & Hooper DC (2016) Serum exosomes and cytokines promote a T-helper cell type 2 environment in the peripheral blood of glioblastoma patients. *Neuro Oncol* **18**, 206–215.

93 Ciregia F, Urbani A & Palmisano G (2017) Extracellular vesicles in brain tumors and neurodegenerative diseases. *Front Mol Neurosci* **10**, 276.

94 Graner MW, Cumming RI & Bigner DD (2007) The heat shock response and chaperones/heat shock proteins in brain tumors: surface expression, release, and possible immune consequences. *J Neurosci* **27**, 11214–11227.

95 Akers JC, Ramakrishnan V, Kim R, Phillips S, Kaimal V, Mao Y, Hua W, Yang I, Fu CC, Nolan J *et al.* (2015) miRNA contents of cerebrospinal fluid extracellular vesicles in glioblastoma patients. *J Neurooncol* **123**, 205–216.

96 Saadatpour L, Fadaee E, Fadaei S, Nassiri Mansour R, Mohammadi M, Mousavi SM, Goodarzi M, Verdi J & Mirzaei H (2016) Glioblastoma: exosome and microRNA as novel diagnosis biomarkers. *Cancer Gene Ther* **23**, 415–418.

97 Manterola L, Guruceaga E, Gallego Perez-Larraya J, Gonzalez-Huarriz M, Jauregui P, Tejada S, Diez-Valle R, Segura V, Sampron N, Barrena C *et al.* (2014) A small noncoding RNA signature found in exosomes of GBM patient serum as a diagnostic tool. *Neuro Oncol* **16**, 520–527.
98 Kanemoto S, Nitani R, Murakami T, Kaneko M, Asada R, Matsuhisa K, Saito A & Imaizumi K (2016) Multivesicular body formation enhancement and exosome release during endoplasmic reticulum stress. *Biochem Biophys Res Commun* **480**, 166–172.
99 Mahadevan NR, Anufreichik V, Rodvold JJ, Chiu KT, Sepulveda H & Zanetti M (2012) Cell-extrinsic effects of tumor ER stress imprint myeloid dendritic cells and impair CD8(+) T cell priming. *PLoS One* **7**, e51845.
100 Mahadevan NR, Rodvold J, Sepulveda H, Rossi S, Drew AF & Zanetti M (2011) Transmission of endoplasmic reticulum stress and pro-inflammation from tumor cells to myeloid cells. *Proc Natl Acad Sci USA* **108**, 6561–6566.
101 Zanetti M (2013) Cell-extrinsic effects of the tumor unfolded protein response on myeloid cells and T cells. *Ann N Y Acad Sci* **1284**, 6–11.
102 Rodvold JJ, Chiu KT, Hiramatsu N, Nussbacher JK, Galimberti V, Mahadevan NR, Willert K, Lin JH & Zanetti M (2017) Intercellular transmission of the unfolded protein response promotes survival and drug resistance in cancer cells. *Sci Signal* **10**, pii: eaah7177.
103 Zhang H, Yue Y, Sun T, Wu X & Xiong S (2017) Transmissible endoplasmic reticulum stress from myocardiocytes to macrophages is pivotal for the pathogenesis of CVB3-induced viral myocarditis. *Sci Rep* **7**, 42162.
104 Urra H & Hetz C (2014) A novel ER stress-independent function of the UPR in angiogenesis. *Mol Cell* **54**, 542–544.
105 Pereira ER, Liao N, Neale GA & Hendershot LM (2010) Transcriptional and post-transcriptional regulation of proangiogenic factors by the unfolded protein response. *PLoS One* **5**, pii: e12521.
106 Drogat B, Auguste P, Nguyen DT, Bouchecareilh M, Pineau R, Nalbantoglu J, Kaufman RJ, Chevet E, Bikfalvi A & Moenner M (2007) IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo. *Cancer Res* **67**, 6700–6707.
107 Wang JM, Qiu Y, Yang ZQ, Li L & Zhang K (2017) Inositol-requiring enzyme 1 facilitates diabetic wound healing through modulating microRNAs. *Diabetes* **66**, 177–192.
108 Wang Y, Alam GN, Ning Y, Visioli F, Dong Z, Nor JE & Polverini PJ (2012) The unfolded protein response induces the angiogenic switch in human tumor cells through the PERK/ATF4 pathway. *Cancer Res* **72**, 5396–5406.
109 Blais JD, Addison CL, Edge R, Falls T, Zhao H, Wary K, Koumenis C, Harding HP, Ron D, Holcik M *et al.* (2006) Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. *Mol Cell Biol* **26**, 9517–9532.
110 Philippe C, Dubrac A, Quelen C, Desquesnes A, Van Den Berghe L, Segura C, Filleron T, Pyronnet S, Prats H, Brousset P *et al.* (2016) PERK mediates the IRES-dependent translational activation of mRNAs encoding angiogenic growth factors after ischemic stress. *Sci Signal* **9**, ra44.
111 Ozawa K, Tsukamoto Y, Hori O, Kitao Y, Yanagi H, Stern DM & Ogawa S (2001) Regulation of tumor angiogenesis by oxygen-regulated protein 150, an inducible endoplasmic reticulum chaperone. *Cancer Res* **61**, 4206–4213.
112 Karali E, Bellou S, Stellas D, Klinakis A, Murphy C & Fotsis T (2014) VEGF Signals through ATF6 and PERK to promote endothelial cell survival and angiogenesis in the absence of ER stress. *Mol Cell* **54**, 559–572.
113 Visioli F, Wang Y, Alam GN, Ning Y, Rados PV, Nor JE & Polverini PJ (2014) Glucose-regulated protein 78 (Grp78) confers chemoresistance to tumor endothelial cells under acidic stress. *PLoS One* **9**, e101053.
114 Han KS, Li N, Raven PA, Fazli L, Frees S, Ettinger S, Park KC, Hong SJ, Gleave ME & So AI (2015) Inhibition of endoplasmic reticulum chaperone protein glucose-regulated protein 78 potentiates anti-angiogenic therapy in renal cell carcinoma through inactivation of the PERK/eIF2alpha pathway. *Oncotarget* **6**, 34818–34830.
115 Ruan Q, Han S, Jiang WG, Boulton ME, Chen ZJ, Law BK & Cai J (2011) alphaB-crystallin, an effector of unfolded protein response, confers anti-VEGF resistance to breast cancer via maintenance of intracrine VEGF in endothelial cells. *Mol Cancer Res* **9**, 1632–1643.
116 Cubillos-Ruiz JR, Bettigole SE & Glimcher LH (2017) Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. *Cell* **168**, 692–706.
117 Shi W, Liao Y, Willis SN, Taubenheim N, Inouye M, Tarlinton DM, Smyth GK, Hodgkin PD, Nutt SL & Corcoran LM (2015) Transcriptional profiling of mouse B cell terminal differentiation defines a signature for antibody-secreting plasma cells. *Nat Immunol* **16**, 663–673.
118 Morita S, Villalta SA, Feldman HC, Register AC, Rosenthal W, Hoffmann-Petersen IT, Mehdizadeh M, Ghosh R, Wang L, Colon-Negron K *et al.* (2017) Targeting ABL-IRE1alpha signaling spares

ER-stressed pancreatic beta cells to reverse autoimmune diabetes. *Cell Metab* **25**, 883–897 e8.
119 Bujisic B, De Gassart A, Tallant R, Demaria O, Zaffalon L, Chelbi S, Gilliet M, Bertoni F & Martinon F (2017) Impairment of both IRE1 expression and XBP1 activation is a hallmark of GCB DLBCL and contributes to tumor growth. *Blood* **129**, 2420–2428.
120 Grootjans J, Kaser A, Kaufman RJ & Blumberg RS (2016) The unfolded protein response in immunity and inflammation. *Nat Rev Immunol* **16**, 469–484.
121 Osorio F, Tavernier SJ, Hoffmann E, Saeys Y, Martens L, Vetters J, Delrue I, De Rycke R, Parthoens E, Pouliot P *et al.* (2014) The unfolded-protein-response sensor IRE-1alpha regulates the function of CD8alpha+ dendritic cells. *Nat Immunol* **15**, 248–257.
122 Tavernier SJ, Osorio F, Vandersarren L, Vetters J, Vanlangenakker N, Van Isterdael G, Vergote K, De Rycke R, Parthoens E, van de Laar L *et al.* (2017) Regulated IRE1-dependent mRNA decay sets the threshold for dendritic cell survival. *Nat Cell Biol* **19**, 698–710.
123 Cubillos-Ruiz JR, Silberman PC, Rutkowski MR, Chopra S, Perales-Puchalt A, Song M, Zhang S, Bettigole SE, Gupta D, Holcomb K *et al.* (2015) ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. *Cell* **161**, 1527–1538.
124 Martinon F, Chen X, Lee AH & Glimcher LH (2010) TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. *Nat Immunol* **11**, 411–418.
125 Thevenot PT, Sierra RA, Raber PL, Al-Khami AA, Trillo-Tinoco J, Zarreii P, Ochoa AC, Cui Y, Del Valle L & Rodriguez PC (2014) The stress-response sensor chop regulates the function and accumulation of myeloid-derived suppressor cells in tumors. *Immunity* **41**, 389–401.
126 Nakagawa H, Umemura A, Taniguchi K, Font-Burgada J, Dhar D, Ogata H, Zhong Z, Valasek MA, Seki E, Hidalgo J *et al.* (2014) ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. *Cancer Cell* **26**, 331–343.
127 Goodall JC, Wu C, Zhang Y, McNeill L, Ellis L, Saudek V & Gaston JS (2010) Endoplasmic reticulum stress-induced transcription factor, CHOP, is crucial for dendritic cell IL-23 expression. *Proc Natl Acad Sci USA* **107**, 17698–17703.
128 Kim S, Joe Y, Kim HJ, Kim YS, Jeong SO, Pae HO, Ryter SW, Surh YJ & Chung HT (2015) Endoplasmic reticulum stress-induced IRE1alpha activation mediates cross-talk of GSK-3beta and XBP-1 to regulate inflammatory cytokine production. *J Immunol* **194**, 4498–4506.
129 Shanware NP, Bray K, Eng CH, Wang F, Follettie M, Myers J, Fantin VR & Abraham RT (2014) Glutamine deprivation stimulates mTOR-JNK-dependent chemokine secretion. *Nat Commun* **5**, 4900.
130 Liu J, Ibi D, Taniguchi K, Lee J, Herrema H, Akosman B, Mucka P, Salazar Hernandez MA, Uyar MF, Park SW *et al.* (2016) Inflammation improves glucose homeostasis through IKKbeta-XBP1s interaction. *Cell* **167**, 1052–1066 e18.
131 Shkoda A, Ruiz PA, Daniel H, Kim SC, Rogler G, Sartor RB & Haller D (2007) Interleukin-10 blocked endoplasmic reticulum stress in intestinal epithelial cells: impact on chronic inflammation. *Gastroenterology* **132**, 190–207.
132 Zhang K, Shen X, Wu J, Sakaki K, Saunders T, Rutkowski DT, Back SH & Kaufman RJ (2006) Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* **124**, 587–599.
133 Yang L, Calay ES, Fan J, Arduini A, Kunz RC, Gygi SP, Yalcin A, Fu S & Hotamisligil GS (2015) METABOLISM. S-Nitrosylation links obesity-associated inflammation to endoplasmic reticulum dysfunction. *Science* **349**, 500–506.
134 Robblee MM, Kim CC, Porter Abate J, Valdearcos M, Sandlund KL, Shenoy MK, Volmer R, Iwawaki T & Koliwad SK (2016) Saturated fatty acids engage an IRE1alpha-dependent pathway to activate the NLRP3 inflammasome in myeloid cells. *Cell Rep* **14**, 2611–2623.
135 Halbleib K, Pesek K, Covino R, Hofbauer HF, Wunnicke D, Hanelt I, Hummer G & Ernst R (2017) Activation of the unfolded protein response by lipid bilayer stress. *Mol Cell* **67**, 673–684 e8.
136 Bronner DN, Abuaita BH, Chen X, Fitzgerald KA, Nunez G, He Y, Yin XM & O'Riordan MX (2015) Endoplasmic reticulum stress activates the inflammasome via NLRP3- and caspase-2-driven mitochondrial damage. *Immunity* **43**, 451–462.
137 Galluzzi L, Buque A, Kepp O, Zitvogel L & Kroemer G (2017) Immunogenic cell death in cancer and infectious disease. *Nat Rev Immunol* **17**, 97–111.
138 Panaretakis T, Kepp O, Brockmeier U, Tesniere A, Bjorklund AC, Chapman DC, Durchschlag M, Joza N, Pierron G, van Endert P *et al.* (2009) Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. *EMBO J* **28**, 578–590.
139 Raghavan M, Wijeyesakere SJ, Peters LR & Del Cid N (2013) Calreticulin in the immune system: ins and outs. *Trends Immunol* **34**, 13–21.
140 Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, Galluzzi L, Adjemian S, Kepp O, Niso-Santano M *et al.* (2012) An immunosurveillance mechanism controls cancer cell ploidy. *Science* **337**, 1678–1684.

141 Park IA, Heo SH, Song IH, Kim YA, Park HS, Bang WS, Park SY, Jo JH, Lee HJ & Gong G (2016) Endoplasmic reticulum stress induces secretion of high-mobility group proteins and is associated with tumor-infiltrating lymphocytes in triple-negative breast cancer. *Oncotarget* **7**, 59957–59964.
142 Cook KL, Soto-Pantoja DR, Clarke PA, Cruz MI, Zwart A, Warri A, Hilakivi-Clarke L, Roberts DD & Clarke R (2016) Endoplasmic reticulum stress protein GRP78 modulates lipid metabolism to control drug sensitivity and antitumor immunity in breast cancer. *Cancer Res* **76**, 5657–5670.
143 Cho JA, Lee AH, Platzer B, Cross BC, Gardner BM, De Luca H, Luong P, Harding HP, Glimcher LH, Walter P *et al.* (2013) The unfolded protein response element IRE1alpha senses bacterial proteins invading the ER to activate RIG-I and innate immune signaling. *Cell Host Microbe* **13**, 558–569.
144 Eckard SC, Rice GI, Fabre A, Badens C, Gray EE, Hartley JL, Crow YJ & Stetson DB (2014) The SKIV2L RNA exosome limits activation of the RIG-I-like receptors. *Nat Immunol* **15**, 839–845.
145 Nabet BY, Qiu Y, Shabason JE, Wu TJ, Yoon T, Kim BC, Benci JL, DeMichele AM, Tchou J, Marcotrigiano J *et al.* (2017) Exosome RNA unshielding couples stromal activation to pattern recognition receptor signaling in cancer. *Cell* **170**, 352–366 e13.
146 Dassler-Plenker J, Reiners KS, van den Boorn JG, Hansen HP, Putschli B, Barnert S, Schuberth-Wagner C, Schubert R, Tuting T, Hallek M *et al.* (2016) RIG-I activation induces the release of extracellular vesicles with antitumor activity. *Oncoimmunology* **5**, e1219827.
147 Poeck H, Besch R, Maihoefer C, Renn M, Tormo D, Morskaya SS, Kirschnek S, Gaffal E, Landsberg J, Hellmuth J *et al.* (2008) 5′-Triphosphate-siRNA: turning gene silencing and Rig-I activation against melanoma. *Nat Med* **14**, 1256–1263.
148 Hou J, Zhou Y, Zheng Y, Fan J, Zhou W, Ng IO, Sun H, Qin L, Qiu S, Lee JM *et al.* (2014) Hepatic RIG-I predicts survival and interferon-alpha therapeutic response in hepatocellular carcinoma. *Cancer Cell* **25**, 49–63.
149 Besch R, Poeck H, Hohenauer T, Senft D, Hacker G, Berking C, Hornung V, Endres S, Ruzicka T, Rothenfusser S *et al.* (2009) Proapoptotic signaling induced by RIG-I and MDA-5 results in type I interferon-independent apoptosis in human melanoma cells. *J Clin Invest* **119**, 2399–2411.
150 Duewell P, Steger A, Lohr H, Bourhis H, Hoelz H, Kirchleitner SV, Stieg MR, Grassmann S, Kobold S, Siveke JT *et al.* (2014) RIG-I-like helicases induce immunogenic cell death of pancreatic cancer cells and sensitize tumors toward killing by CD8(+) T cells. *Cell Death Differ* **21**, 1825–1837.
151 Su HY, Waldron RT, Gong R, Ramanujan VK, Pandol SJ & Lugea A (2016) The unfolded protein response plays a predominant homeostatic role in response to mitochondrial stress in pancreatic stellate cells. *PLoS One* **11**, e0148999.
152 Harris NLE, Vennin C, Conway JRW, Vine KL, Pinese M, Cowley MJ, Shearer RF, Lucas MC, Herrmann D, Allam AH *et al.* (2017) SerpinB2 regulates stromal remodelling and local invasion in pancreatic cancer. *Oncogene* **36**, 4288–4298.
153 Buganim Y, Madar S, Rais Y, Pomeraniec L, Harel E, Solomon H, Kalo E, Goldstein I, Brosh R, Haimov O *et al.* (2011) Transcriptional activity of ATF3 in the stromal compartment of tumors promotes cancer progression. *Carcinogenesis* **32**, 1749–1757.
154 Hetz C & Saxena S (2017) ER stress and the unfolded protein response in neurodegeneration. *Nat Rev Neurol* **13**, 477–491.
155 Farooqi AA, Li KT, Fayyaz S, Chang YT, Ismail M, Liaw CC, Yuan SS, Tang JY & Chang HW (2015) Anticancer drugs for the modulation of endoplasmic reticulum stress and oxidative stress. *Tumour Biol* **36**, 5743–5752.
156 Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P & Vandenabeele P (2012) Immunogenic cell death and DAMPs in cancer therapy. *Nat Rev Cancer* **12**, 860–875.
157 Rufo N, Garg AD & Agostinis P (2017) The unfolded protein response in immunogenic cell death and cancer immunotherapy. *Trends Cancer* **3**, 643–658.
158 Salaroglio IC, Panada E, Moiso E, Buondonno I, Provero P, Rubinstein M, Kopecka J & Riganti C (2017) PERK induces resistance to cell death elicited by endoplasmic reticulum stress and chemotherapy. *Mol Cancer* **16**, 91.
159 Higa A, Taouji S, Lhomond S, Jensen D, Fernandez-Zapico ME, Simpson JC, Pasquet JM, Schekman R & Chevet E (2014) Endoplasmic reticulum stress-activated transcription factor ATF6alpha requires the disulfide isomerase PDIA5 to modulate chemoresistance. *Mol Cell Biol* **34**, 1839–1849.
160 Bu LJ, Yu HQ, Fan LL, Li XQ, Wang F, Liu JT, Zhong F, Zhang CJ, Wei W, Wang H *et al.* (2017) Melatonin, a novel selective ATF-6 inhibitor, induces human hepatoma cell apoptosis through COX-2 downregulation. *World J Gastroenterol* **23**, 986–998.
161 Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, Tam A, Solow-Cordero DE, Bouley DM, Offner F, Niwa M *et al.* (2011) Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. *Blood* **117**, 1311–1314.
162 Ming J, Ruan S, Wang M, Ye D, Fan N, Meng Q, Tian B & Huang T (2015) A novel chemical, STF-083010, reverses tamoxifen-related drug resistance in

breast cancer by inhibiting IRE1/XBP1. *Oncotarget* **6**, 40692–40703.

163 Mimura N, Fulciniti M, Gorgun G, Tai YT, Cirstea D, Santo L, Hu Y, Fabre C, Minami J, Ohguchi H *et al*. (2012) Blockade of XBP1 splicing by inhibition of IRE1alpha is a promising therapeutic option in multiple myeloma. *Blood* **119**, 5772–5781.

164 Ghosh R, Wang L, Wang ES, Perera BG, Igbaria A, Morita S, Prado K, Thamsen M, Caswell D, Macias H *et al*. (2014) Allosteric inhibition of the IRE1alpha RNase preserves cell viability and function during endoplasmic reticulum stress. *Cell* **158**, 534–548.

165 Axten JM, Medina JR, Feng Y, Shu A, Romeril SP, Grant SW, Li WH, Heerding DA, Minthorn E, Mencken T *et al*. (2012) Discovery of 7-methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-p yrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). *J Med Chem* **55**, 7193–7207.

166 Atkins C, Liu Q, Minthorn E, Zhang SY, Figueroa DJ, Moss K, Stanley TB, Sanders B, Goetz A, Gaul N *et al*. (2013) Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res* **73**, 1993–2002.

167 Palam LR, Gore J, Craven KE, Wilson JL & Korc M (2015) Integrated stress response is critical for gemcitabine resistance in pancreatic ductal adenocarcinoma. *Cell Death Dis* **6**, e1913.

168 Jeon YJ, Kim JH, Shin JI, Jeong M, Cho J & Lee K (2016) Salubrinal-mediated upregulation of eIF2alpha phosphorylation increases doxorubicin sensitivity in MCF-7/ADR cells. *Mol Cells* **39**, 129–135.

Résumé

Plusieurs arguments de la littérature suggèrent l'importance de l'alimentation dans le développement tumoral et l'efficacité des traitements anti-cancereux. Dans différents modèles animaux, la restriction calorique (CR) supprime la prolifération des cellules tumorales et les sensibilise aux thérapies ciblées. Par conséquent, des approches non-pharmacologiques comme la restriction calorique ont un intérêt grandissant en clinique.

Considérant l'addiction des cellules tumorales aux nutriments, nous nous sommes demandé quels macronutriments pouvaient avoir des propriétés anticancéreuses. A partir d'un modèle murin de lymphomes B (modèle transgénique Eμ-Myc) nous avons testé l'impact de deux régimes alimentaires : l'un pauvre en glucides (Low CHO, 25% de réduction en glucides) et l'autre pauvre en protéines (Low PROT, 25% de réduction en protéines). Des souris syngéniques C57BL/6 ont été injectées par voie intraveineuse avec des cellules primaires Eμ-Myc. Malgré un apport alimentaire équivalent entre les groupes, nous avons observé que le régime pauvre en protéines augmente la survie globale des souris C57BL/6 développant un lymphome B Eμ-Myc. De manière intéressante, nous avons démontré que cet effet pro-survie est dépendant du système immunitaire. En effet, la déplétion des cellules T $CD8^+$ ou l'utilisation d'un modèle murin immunodéficient NSG (NOD-SCID il2rγ), empêche l'effet bénéfique du régime pauvre en protéines sur le développement tumoral. Nous avons reproduit et étendu nos observations en utilisant des lignées modèles de cancéreuses colorectaux (CT26) et de mélanome (B16) injectée dans des souris syngéniques, immunocompétente.

Les cellules tumorales étant fortement dépendantes des nutriments, nous avons émis l'hypothèse qu'un régime pauvre en protéines pourrait induire un stress du réticulum endoplasmique (RE) dans ces dernières. En effet, nous avons observé une augmentation des protéines impliquées dans la signalisation du RE : CHOP et sXBP1. Par conséquent, nous avons traité les souris nourries en régime pauvre en protéines avec deux inhibiteurs du stress du RE : TUDCA, inhibiteur générique et MKC4485 qui cible l'activité ribonucléase d'IRE1. Dans les deux cas, ces inhibiteurs ont bloqué l'effet du régime faible en protéines sur le développement tumoral et l'infiltration des T $CD8^+$ au sein de la tumeur. Pour s'affranchir, des potentiels effets secondaires des inhibiteurs chimiques, nous avons invalidé IRE1 dans la lignée CT26 et nous avons obtenus des résultats similaires, démontrant que la voie IRE1 dans les cellules tumorales est une voie centrale dans la réponse immunitaire anticancéreuse induite par un régime pauvre en protéines. En outre, nous avons découvert que l'activation de RIG-I est un événement en aval de l'activation d'IRE1 et que, par analyse bio-informatique nous avons pu corréler une signature IRE1 à une infiltration immunitaire élevée et à une immunogénicité accrue du cancer chez les patients atteints de mélanome, glioblastome et cancer colorectal. De ce fait, nous avons démontré que la réponse du système immunitaire induite par un régime pauvre en protéines est une conséquence de l'activation accrue de IRE1 dans les cellules cancéreuses.

Plusieurs arguments de la littérature suggèrent l'importance de l'alimentation dans le développement tumoral et l'efficacité des traitements anti-cancereux. Dans différents modèles animaux, la restriction calorique (CR) supprime la prolifération des cellules tumorales et les sensibilise aux thérapies ciblées. Par conséquent, des approches non-pharmacologiques comme la restriction calorique ont un intérêt grandissant en clinique.

Considérant l'addiction des cellules tumorales aux nutriments, nous nous sommes demandé quels macronutriments pouvaient avoir des propriétés anticancéreuses. A partir d'un modèle murin de lymphomes B (modèle transgénique Eμ-Myc) nous avons testé l'impact de deux régimes alimentaires : l'un pauvre en glucides (Low CHO, 25% de réduction en glucides) et l'autre pauvre en protéines (Low PROT, 25% de réduction en protéines). Des souris syngéniques C57BL/6 ont été injectées par voie intraveineuse avec des cellules primaires Eμ-Myc. Malgré un apport alimentaire équivalent entre les groupes, nous avons observé que le régime pauvre en protéines augmente la survie globale des souris C57BL/6 développant un lymphome B Eμ-Myc. De manière intéressante, nous avons démontré que cet effet pro-survie est dépendant du système immunitaire. En effet, la déplétion des cellules T $CD8^+$ ou l'utilisation d'un modèle murin immunodéficient NSG (NOD-SCID il2rγ), empêche l'effet bénéfique du régime pauvre en protéines sur le développement tumoral. Nous avons reproduit et étendu nos observations en utilisant des lignées modèles de cancéreuses colorectaux (CT26) et de mélanome (B16) injectée dans des souris syngéniques, immunocompétente.

Les cellules tumorales étant fortement dépendantes des nutriments, nous avons émis l'hypothèse qu'un régime pauvre en protéines pourrait induire un stress du réticulum endoplasmique (RE) dans ces dernières. En effet, nous avons observé une augmentation des protéines impliquées dans la signalisation du RE : CHOP et sXBP1. Par conséquent, nous avons traité les souris nourries en régime pauvre en protéines avec deux inhibiteurs du stress du RE : TUDCA, inhibiteur générique et MKC4485 qui cible l'activité ribonucléase d'IRE1. Dans les deux cas, ces inhibiteurs ont bloqué l'effet du régime faible en protéines sur le développement tumoral et l'infiltration des T $CD8^+$ au sein de la tumeur. Pour s'affranchir, des potentiels effets secondaires des inhibiteurs chimiques, nous avons invalidé IRE1 dans la lignée CT26 et nous avons obtenus des résultats similaires, démontrant que la voie IRE1 dans les cellules tumorales est une voie centrale dans la réponse immunitaire anticancéreuse induite par un régime pauvre en protéines. En outre, nous avons découvert que l'activation de RIG-I est un événement en aval de l'activation d'IRE1 et que, par analyse bio-informatique nous avons pu corréler une signature IRE1 à une infiltration immunitaire élevée et à une immunogénicité accrue du cancer chez les patients atteints de mélanome, glioblastome et cancer colorectal. De ce fait, nous avons démontré que la réponse du système immunitaire induite par un régime pauvre en protéines est une conséquence de l'activation accrue de IRE1 dans les cellules cancéreuses.