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Abir Zebian

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POUR OBTENIR LE GRADE DE
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**Etude du facteur de réparation de l'ADN,
Xeroderma pigmentosum du groupe C (XPC),
dans les cellules souches hématopoïétiques**

Sous la direction de: Frédéric, MAZURIER

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Soutenue le 12 Décembre 2014

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Titre : Etude du facteur de réparation de l'ADN, Xeroderma pigmentosum du groupe C (XPC), dans les cellules souches hématopoïétiques

Résumé : Les dommages de l'ADN peuvent s'accumuler dans les cellules souches hématopoïétiques (CSH) suite aux stress externes ou métaboliques et perturber leur fonctionnement et/ou leur maintien. La réparation par excision de nucléotides (NER), initiée par l'arrêt de la transcription (TCR) ou par la reconnaissance de distorsions des régions non transcrites (GGR) de l'ADN, est nécessaire à l'hématopoïèse à long terme. XPC, un facteur clé du système GGR, participe à d'autres réponses au stress oxydatif. Le laboratoire a montré que la perte de XPC provoque l'accumulation de mutations, un stress métabolique et la carcinogenèse. Notre objectif est d'évaluer son expression et son rôle dans le maintien et la différenciation des CSH. Nos résultats montrent qu'il est plus exprimé dans les cellules immatures CD34⁺ que dans les CD34⁻ matures. Aussi, XPC apparaît sous trois poids moléculaires différents certainement liés à des modifications post-traductionnelles. Son extinction par ARN interférence n'affecte ni la prolifération ni la capacité progénitrice *in vitro* des cellules CD34⁺. Cependant, les cellules déficientes implantées chez des souris immunodéficientes disparaissent progressivement suggérant une perte des CSH ou de leur capacité de différenciation. Postulant que les mutations s'accumulent avec le temps, nous avons étudié l'hématopoïèse chez des souris déficientes en XPC jeunes et âgées. Les différences décrites dans l'hématopoïèse chez les individus jeunes et âgés sont retrouvées mais, de manière surprenante, aucune différence entre les animaux sauvages et mutés quelque soit l'âge ou le stress génotoxique n'est observée. Les résultats obtenus sur les cellules humaines démontrent un rôle potentiel de XPC dans l'hématopoïèse, mais de nouvelles investigations sont nécessaires pour mieux comprendre les mécanismes impliqués, et la possible participation de XPC dans la leucémogenèse.

Mots clés: XPC, CSH, KO, CD34, Maintenance, Vieillesse

Title: Study of DNA repair factor Xeroderma pigmentosum group C (XPC) in hematopoietic stem cells

Abstract: DNA damage may accumulate in hematopoietic stem cells (HSC) due to external or metabolic stresses, leading to perturbation in their function and/or maintenance. Nucleotide excision repair (NER), initiated in the DNA by the stop of transcription (TCR) or by the recognition of distortions in transcribed regions (GGR), is necessary for long-term hematopoiesis. XPC, a key factor in GGR, is implicated in oxidative stress. The laboratory has demonstrated that XPC loss leads to the accumulation of mutations, metabolic stress and carcinogenesis. Our objective is to evaluate XPC expression and its role in HSC maintenance and differentiation. Results showed that XPC is highly expressed in immature CD34⁺ cells compared to mature CD34⁻ cells. In addition, XPC appeared with three different molecular weights, certainly linked to post-translational modifications. XPC silencing by shRNA did not affect the proliferation or the progenitor ability of CD34⁺ cells *in vitro*. However, deficient cells transplanted in immunodeficient mice disappeared progressively, suggesting the loss of HSCs or their differentiation capacity. Postulating that mutations accumulate with time, we have studied hematopoiesis in young and aged XPC deficient mice. Differences described in young and aged hematopoiesis systems were found but, surprisingly, no difference was observed between wild type and mutant mice at any age or genotoxic stress. Data from human cells demonstrate a potential role for XPC in HSC but new investigations are necessary to better understand the mechanisms implicated and if XPC may participate in leukemogenesis.

Keywords: XPC, HSC, KO, CD34, Maintenance, Aging

Unité de recherche

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List of Abbreviations

4NQO	:	4-nitroquinoline-1-oxide
6-4PPs	:	pyrimidine-(6,4)-pyrimidone products
AAAF	:	N-acetoxy-acetylaminofluorine
AGM	:	Aorta, Gonads, Mesonephros
AHR	:	Aryl hydrocarbon receptor
Akt1	:	v-akt murine thymoma viral oncogene homolog 1
ALDH	:	Aldehyde dehydrogenases
AML	:	Acute myelogenous leukemia
AP	:	Apurinic/ apyrimidic
APE1	:	AP endonuclease 1
ASC	:	Adult stem cells
ATM	:	Ataxia telangiectasia mutated
ATR	:	Ataxia telangiectasia mutated and Rad3-related
Bala	:	Balanced
BANF1	:	Barrier to autointegration factor 1
BER	:	Base excision repair
BFU-E	:	Burstforming unit erythroid
BHD1	:	β -hairpin domain
BLM	:	Bloom syndrome, RecQ helicase like
BM	:	Bone marrow
BMT	:	Bone marrow transplantation
BPDE	:	Benzo[a]pyrenedilepoxide
BRCA	:	Breast cancer 1, early onset
CAF-1	:	Chromatin assembly factor 1
CD	:	Cluster of differentiation
CETN2	:	Centrin 2
CFC	:	Colony forming cell
CFU-E	:	Colony forming unit erythroid

CFU-GEMM	:	CFU- Granulocyte /Erythroid /Macrophage /Megakaryocyte
CFU-GM	:	Colony Forming Unit- Granulocyte /Macrophage
CHRAC1	:	Chromatin accessibility complex 1
CLP	:	Common lymphoid progenitors
CML	:	Chronic myelogenous leukemia
CML	:	Chronic myeloid leukemia
CMP	:	Common myeloid progenitors
COFS	:	Cerebrooculo-facial skeletal syndrome
CPPs	:	Cyclobutane pyrimidine dimer
CS	:	Cockayne syndrome
CSA	:	Cockayne syndrome complementation group A
CSB	:	Cockayne syndrome complementation group B
CSC	:	Cancer stem cell
CtBP	:	C-Terminal Binding Protein
CtIP	:	CTBP-interacting protein
Cul4	:	Cullin 4
CXCL12	:	Chemokine (C-X-C motif) ligand 12
DAR	:	Differentiation associated repair
DDB	:	Damage-specific DNA binding protein
DDR	:	DNA damage response
DEHP	:	Diethylhexyl phthalate
DNA-PK	:	DNA-protein kinase
DSB	:	Double-strand breaks
ESCs	:	Embryonic stem cells
Exo1	:	Exonuclease 1
FEN	:	Flap endonuclease 1
FSC	:	Fetal stem cell
GGR	:	Global genome repair
GMP	:	Granulocyte /Macrophage progenitor
Gpx	:	Glutathione peroxidase
GVHD	:	Graft-versus-host disease

HAS	:	Human serum albumin
HIF-1	:	Hypoxia-inducible factor-1
HPC	:	Hematopoietic progenitor cell
HPRT	:	Hypoxanthine guanine phosphor-ribosyl transferase
HR	:	Homologous recombination
HR23B	:	Human RAD23 homolog B
HRE	:	Hypoxia response element
HRE	:	Hypoxia response element
HSC	:	Hematopoietic stem cells
HSPC	:	Hematopoietic stem/ progenitor cell
ICM	:	Inner cell mass
IDL	:	Insertion deletion loops
iPSCs	:	Induced Pluripotent stem cells
IR	:	Ionizing radiation
IT-HSC	:	Intermediate HSC
KD	:	Knockdown
KO	:	Knockout
L/M	:	Lymphoid/myeloid
LDF	:	Leukodepletion filter
LMPP	:	Lymphoid-primed MPP
LT-HSC	:	Long-term HSC
LTR	:	Long term repopulation
Ly-bi	:	Lymphoid biased
Mdr1	:	Multidrug resistance 1
MECP2	:	Methyl-CpG-binding protein 2
MEF	:	Mouse embryonic fibroblast
MEP	:	Megakaryocyte /Erythroid progenitors
MLH	:	MutL homolog
MLP	:	Myeloid lymphoid progenitor
MMR	:	Mismatch repair
MPP	:	Multipotent progenitor cell

MRN	:	Mre11-Rad50-Nbs1
MSCs	:	Mesenchymal stem cells
MSH	:	MutS homolog
mtDNA	:	Mitochondrial DNA
MutLa	:	Heterodimer of MLH1 and PMS2
MutSa	:	Heterodimer of MSH2 and MSH6
MutS β	:	Heterodimer of MSH2 and MSH3
My-bi	:	Myeloid-biased
MyBT	:	Granulocyte / macrophage/ B-lymphocyte/ T-lymphocyte
MyE	:	Myeloid erythroid
NER	:	Nucleotide excision repair
NHEJ	:	Non homologous end joining
NK	:	Natural killer
NOG	:	NOD/ scid/ Il-2R γ
NOX	:	NADPH oxidase
NSC	:	Neural stem cells
OB-fold	:	Oligonucleotide/ oligosaccharide-binding fold
Oct4	:	Octamer-binding transcription factor 4
OGG1	:	8-Oxoguanine glycosylase
OTUD4	:	OTU domain-containing protein 4
PB	:	Peripheral blood
PBSCT	:	Peripheral blood stem cell transplantation
PCNA	:	Proliferation cell nuclear antigen
PDC	:	Paired daughter cell
PHD	:	Prolyl hydroxylase
PKcs	:	Protein kinase catalytic subunit
PMS	:	Postmeiotic Segregation Increased 2
RBC	:	Red blood cells
rCMP	:	CMP with reconstituting potential
RFC	:	Replication factor C
Rho-123	:	Rhodamine-123

RNF111	:	Ring Finger Protein 111
Roc1	:	Rice outmost cell-specific gene 1
ROS	:	Reactive oxygen species
RPA	:	Replication protein A
SC	:	Stem cell
SCF	:	Stem cell factor
SLAM	:	Signaling Lymphocyte activation molecule
SlamF1	:	SLAM family member 1
SMAP1	:	Stromal membrane-associated protein 1
SMUG1	:	Single-strand selective monofunctional uracil DNA glycosylase
SNOs	:	Spindle-shaped N-cadherin-expressing osteoblasts
SNPs	:	Single nucleotide polymorphism
SOD	:	superoxide dismutase
Sox2	:	SRY (sex determining region Y)-box 2
Sp1	:	Specificity protein 1
SR1	:	Stem regenin1
SSPB	:	Steady state peripheral blood
ST-HSC	:	Short-term HSC
SWI/SNF	:	SWItch/Sucrose NonFermentable
TALE	:	Transcription activator-like effector
TCR	:	Transcription coupled repair
TDG	:	Thymine DNA glycosylase
TFIIH	:	Transcription factor II Human
Tg	:	Thymine glycol
TGD	:	Transglutaminase homology domain
TLS	:	Translesion synthesis
TPO	:	Thrombopoietin
TSS	:	Transcription start site
TTD	:	Trichothiodystrophy
UCB	:	Umbilical cord blood
UDS	:	Unscheduled DNA synthesis

USP11	:	Ubiquitin carboxyl-terminal hydrolase 11
UV	:	Ultraviolet
VHL	:	Von Hippel Lindau
WIP1	:	Wild type p53-induced phosphatase 1
WRAP53	:	WD repeat containing, antisense to TP53
XLF	:	XRCC4-like factor
XP	:	Xeroderma pigmentosum
XPA	:	Xeroderma pigmentosum complement group A
XPC	:	Xeroderma pigmentosum complement group C
XPB	:	Xeroderma pigmentosum complement group D
XP-V	:	XP variant
XRCC	:	X-ray repair cross-complementing protein 1
ZCCHC6	:	Zinc finger, CCHC domain containing 6
ZNF512B	:	Zinc finger protein 512B

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Preamble

The integrity of DNA double helix, known to harbor the whole genetic material, is fundamental for the survival of cells, tissues and organs. Throughout life, DNA integrity is continuously threatened by abundant stress factors such as ultraviolet radiation, chemical exposure and endogenous lesions created by the ongoing metabolic processes inside the cell. The accumulation of DNA damage in the cell is indeed hazardous and can possibly trigger cell death, aging-related diseases and increased risk of cancer. Thereby, living cells are shielded with several DNA damage repair pathways amongst them is nucleotide excision repair (NER). NER is a versatile DNA repair pathway which repairs broad spectrum of bulky DNA lesions and helix-distorting damages. NER is divided into two subpathways: global genome repair (GGR) and transcription coupled repair (TCR) which differ in the DNA damage recognition step. After DNA damage recognition, both subpathways converge along the same process which involves DNA damage unwinding, DNA damage incision, DNA synthesis and ligation. Indeed, defects in any of the genes involved in NER mechanism provoke severe syndromes such as xeroderma pigmentosum (XP), cockayne syndrome and trichothiodystrophy.

XP was the first human NER-deficient disease to be discovered and refers to parchment pigmented skin. It is rare disease characterized by an early onset of cutaneous abnormalities due to a cellular hypersensitivity to UV radiation. Related symptoms include photosensitivity, cutaneous atrophy and telangiectasia, actinic keratosis, malignant skin neoplasms, in addition to ocular and neurological abnormalities. Indeed, there are seven complementation groups that have been identified to be involved in XP disease. These include *XP-A* through *XP-G* that are caused by mutations in genes that code for XPA to XPG proteins plus a variant form XPV that is caused by mutation in a gene that codes for polymerase- η .

Xeroderma pigmentosum group C (XPC) is one of the most common complementation groups of XP. It represents a key weapon used by the cell to defend itself against DNA damage. XPC protein is majorly known as an essential DNA damage recognition protein of the GGR subpathway where it harbors domains that can bind to damaged DNA and repair factors and which is followed by the repair machinery being recruited to eliminate the DNA damage.

Due to its role as a DNA damage sensor, XPC defect has been demonstrated to result in cancer phenotype in humans as well as in mice. However, more recently, XPC has been

linked to function outside of NER since XPC-deficient mice show a divergent tumor spectrum compared to other NER deficient mouse models. XPC has been shown to be involved in the recognition of oxidized bases and base excision repair (BER). Markedly, XPC deficient keratinocytes were reported to become tumorigenic in absence of external stress. Hence, XPC seems to function in the removal of oxidative DNA damage, redox homeostasis and cell cycle control including checkpoint regulation and apoptosis that are potential causes of the observed increased cancer susceptibility in oxygen exposed tissues. Interestingly, several studies reported an implication of XPC in the hematopoietic system, in leukemic cell lines and in leukemic patients. Therefore, these important roles of XPC suggest that it could also have a potential role in hematopoietic stem cell (HSC) maintenance.

HSC reside at the apex of the hematopoietic hierarchy, and due to its self renewal and differentiation characteristic, they are capable of sustaining daily regeneration of mature erythroid, myeloid and lymphoid blood cells throughout the life. Thus, HSC continuously ongoing metabolic processes exposes them to genotoxic environmental and metabolic stresses that could generate mutations and sometime trigger leukemogenesis. Importance of NER in HSC long-term repopulation ability has been demonstrated in the xeroderma pigmentosum group D (XPD)-deficient mice. Yet, XPD is involved in the both GGR and TCR subpathways of NER. Still, until now there has not been any report studying the sole importance of GGR in maintenance of human and murine HSC. Thus, we have started this project with an intention to decouple the two subpathways of NER and to focus on GGR implication in HSC integrity. In consequence, since XPC is an initiator of GGR, we have decided to study its implication on HSC fate, using *in vitro* and *in vivo* model systems. We have divided our project into two parts: human study and mouse study. For the human study, we evaluated XPC knockdown (KD) effect on maintenance of umbilical cord blood (UCB)-derived CD34⁺ cells. For the mouse study, we evaluated *Xpc* knockout (KO) effect on maintenance of bone marrow (BM)-derived HSC and, additionally, we investigated the effect of aging on *Xpc* KO model mice. Indeed, we hope to unravel hidden mechanisms for aging and carcinogenesis which may contribute to improvements at the clinical level.

Overall, my thesis work handle the role of DNA damage sensor, xeroderma pigmentosum group C (XPC), in the long-term maintenance of human hematopoietic stem cells (HSC) in immunodeficient mice. It also handles the role of XPC and aging in the maintenance of murine hematopoiesis with or without stress exposure.

Chapter 1

INTRODUCTION

I. Stem Cells

A. Definition

Stem cells (SC) are heterogeneous population of functionally undifferentiated cells capable of proliferation, production of large number of differentiated functional progeny known as potency, self-renewal, homing to an appropriate growth environment, and regeneration of tissue after injury with flexibility and reversibility in the use of these options (Loeffler and Roeder, 2002). The self-renewal characteristic of SC represents their ability to produce phenocopies of themselves by undergoing symmetric cell division by which a cell gives two identical daughter cells enabling the maintenance of the SC pool. The potency characteristic of SC represents their ability to differentiate into multiple specialized cell types by undergoing asymmetric cell division that will generate two non-identical daughter cells, one with original stemness characteristics and the other becomes a differentiated cell. The asymmetry can occur pre-cell division termed as “divisional asymmetry” or post-cell division termed as “environmental asymmetry” (Wilson and Trumpp, 2006) (**Figure 1**).

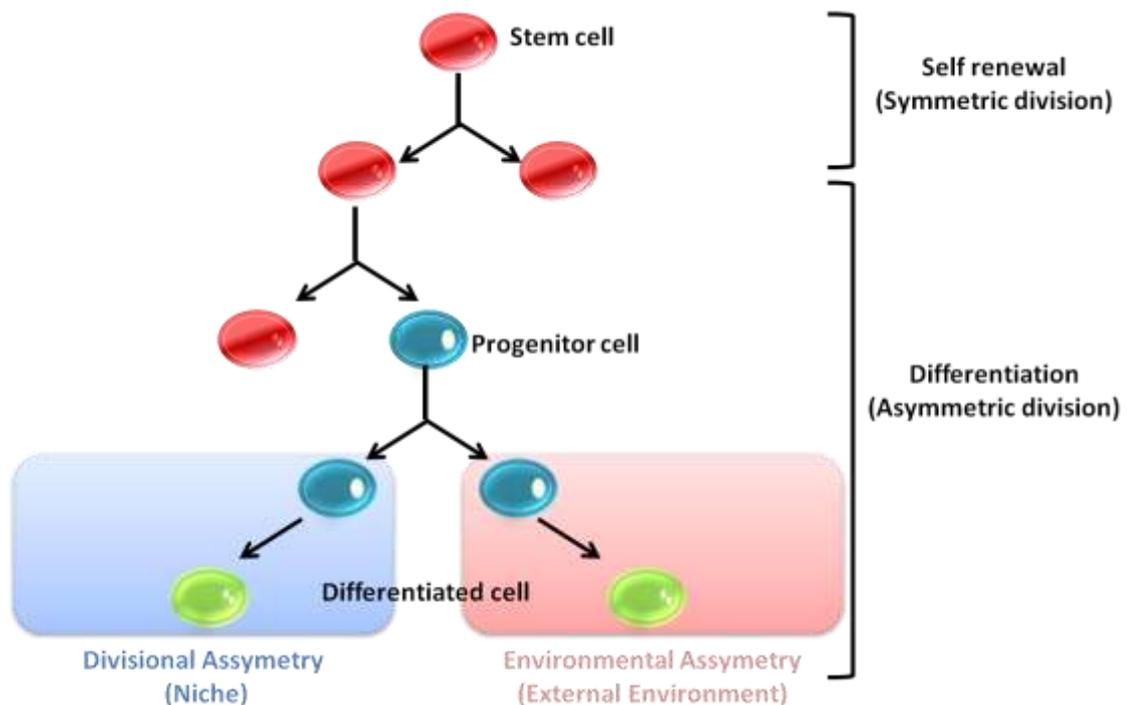


Figure 1. Properties of stem cells. SC produce phenocopies of themselves by their self renewal potential and produce different cell types by their differentiation property. SC self renew by symmetric division and differentiate into mature cells by divisional or environmental asymmetric division. *Figure designed based on information from (Wilson and Trumpp, 2006).*

B. Classification

1. Potency

SC can be classified into four major types according to their potency. These types are the following: totipotent, pluripotent, multipotent and unipotent stem cells (Hui et al., 2011) (Figure 2).

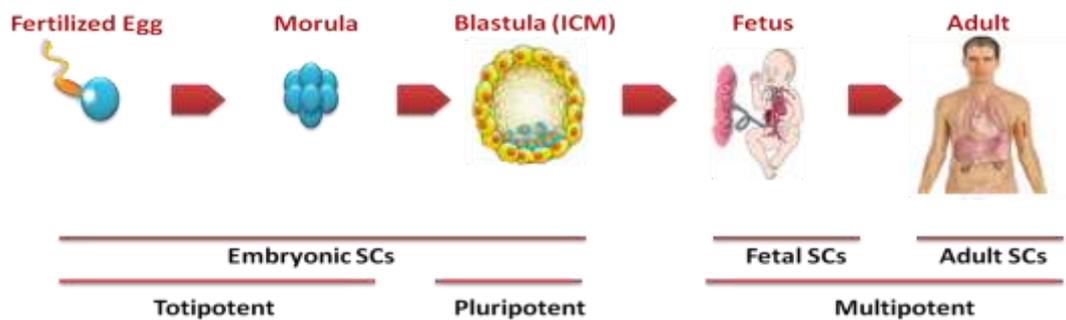


Figure 2. Classification of stem cells. SC can be classified by their potency into totipotent, pluripotent, multipotent, and unipotent. SC can be also classified by their source into embryonic, fetal and adult.

a. Totipotent cells

Totipotent SC are formed from the first divisions of the fertilized egg until the fourth day. They are able of giving rise to all cell types of an organism such as liver, brain, blood or heart cells and are the only cells that permit the full development of an individual. Examples of totipotent cells are zygote, spore and morula.

b. Pluripotent cells

Pluripotent SC are derived from an embryo of five to seven days, and are capable of giving birth to more than 200 cell types representative of all body tissues. These unspecialized SC have the ability to give rise to different types of cells that develop from the three germ layers from which, then, all cells of the body arise. Two examples of pluripotent SC are embryonic stem cells (ESC) and fetal stem cells (FSC). In fact, ESC originate from cells of the inner cell mass (ICM) of the blastocyst. ESC refer to cells that will develop into all tissue types in the body, excluding the placenta (Avasthi et al., 2008; Morrison and Kimble, 2006).

c. Multipotent cells

Multipotent SC, found in fetal and adult tissues, are capable of giving rise to several cell types. These SC have the ability to differentiate into a limited number of specialized cell types. In fact, most adult tissues have multipotent stem cells that can produce a limited range

of differentiated cell lineages. Examples of multipotent SC are mesenchymal stem cells (MSC) and hematopoietic stem cells (HSC).

d. Unipotent cells

Unipotent SC are able to self-renew but have a very limited ability to differentiate relative to other SC. These cells are committed progenitors present at the bottom of the hierarchical tree, which can generate one specific cell type (Inaba and Yamashita, 2012). These SC have unlimited reproductive capabilities, but can only differentiate into a single cell type. An example of unipotent SC is skin cells that must undergo cell division in order to replace damaged cells.

C. Sources of SC

SC can also be classified according to their developmental versatility or source of origin into embryonic, fetal, adult stem cells (**Figure 2**).

1. Embryonic stem cells (ESC)

ESC are pluripotent and are derived four to five days after fertilization from the inner cell mass of the mouse embryonic blastocyst which is an early 32-cell stage embryo. They are able to differentiate into all derivatives of three primary germ layers and they are capable of propagating themselves indefinitely, under defined conditions (Ying et al., 2003). In order to preserve the stemness of ESC, various proteins such as Oct4 and Nanog are implicated. In 1981, in an attempt to generate ESC, Evans and Kaufman cultured mouse embryos in the uterus (Evans and Kaufman, 1981) while Martin and his team demonstrated the possibility of culturing embryos *in vitro* (Martin, 1981). In 1998, Thomson et al. were able to successfully isolate and grow human ESC in cell culture (Thomson et al., 1998). In 2008, researchers have succeeded to cure mice with acute liver injury by transplanting pre-treated human ESC that differentiated into hepatocytes and repopulated the damaged liver (Agarwal et al., 2008). In 2009, the world's first human ESC trial received approval by FDA wherein oligodendrocytes derived from human ESC were transplanted into spinal cord-injured. In October 2010, the first patient was enrolled and ESC administered at Shepherd Center in Atlanta (Villa et al., 2000). Since ESC represent an unlimited source of any cell type, they could be used for "replacement" therapy to provide a possible cure for many conditions. However, the fact that the fertilized egg must be sacrifice to extract ESC, the difficulty in controlling the

proliferation and differential potential of ESC, the potential risk of teratomas upon the graft, renders the therapeutic use of ESC ethically debatable and largely unacceptable.

2. Fetal stem cells (FSC)

Fetal stem cells are primitive cell types found in the organs of fetuses after the blastocyst stage but before birth. Fetal stem cells are capable to differentiate into two types of stem cells: pluripotent stem cells and hematopoietic stem cells. Fetal blood, placenta and umbilical cord are rich sources of fetal hematopoietic stem cells. Fetal neural stem cells found in the fetal human brain were shown to differentiate into both neurons and glial cells (Villa et al., 2000). Human fetal liver progenitor cells have shown enormous proliferation and differentiation capacity to generate mature hepatocytes after transplantation in immunodeficient animals (Soto-Gutierrez et al., 2009).

3. Adult stem cells (ASC)

ASC are tissue-specific stem cells found inside a tissue or organ and are capable of forming the cells needed by that tissue. They can originate from endoderm, mesoderm and ectoderm. For example, pulmonary epithelium, gastrointestinal tract, pancreatic, mammary and prostatic gland, ovarian and testicular SC are of endodermal origin, while hematopoietic, mesenchymal and bone marrow SC are of mesodermal origin and neural, skin and ocular SC are of ectodermal origin. ASC have limited potential, being multipotent, compared to ESC and FSC because of their stage of development and serve mainly in local tissue repair and regeneration. ASC exist in a differentiated tissue and are capable, under certain circumstances, to become specialized. In this context, neural stem cells (NSC) have the ability to differentiate to neuronal and glial cells, MSC have the ability to give rise to a variety of connective tissues *in vitro* and *in vivo* such as bone, cartilage, adipose, and muscle tissues, and HSC have the ability to differentiate into all types of blood cells. In addition, therapeutic use of ASC is ethically acceptable worldwide and is the subject of extensive modern clinical research (Bongso and Tan, 2005).

D. Function of SC

SC are implicated majorly in organogenesis during embryonic development in which they act in specification and morphogenesis of tissues. Stem cells also function in homeostasis during adult life in which they act in maintenance and repair of adult tissues by replacing cells lost during normal tissue turnover or after injury (Goessler et al., 2006; Mitalipov and Wolf, 2009). In this context, tissue-specific SC are found in many highly regenerative organs, such as blood, skin, and the digestive tract but they are also found in non-renewing organs such as muscle, where they allow repair after tissue damage. For instance, the loss of tissue-specific SC leads to perturbation of the homeostatic reserve and hence promotes aging (Schlessinger and Van Zant, 2001; Van Zant and Liang, 2003). Furthermore, tissue-specific SC have major implications in carcinogenesis. Due to their longevity and unlimited capacity for cell division, the risk of accumulating multiple mutations is highly increased. In addition, the mechanisms involved in their quiescence makes them more prone to resist chemotherapy when transformed and hence a potential source of tumor recurrence (Barnes and Melo, 2006; Reya et al., 2001).

II. Hematopoietic Stem Cells (HSC)

A. Hematopoiesis

Hematopoiesis proceeds through an organized developmental hierarchy initiated by HSC that give rise to progressively more committed progenitors and eventually terminally differentiated blood cells (Bryder et al., 2006). In the adult, it originates in the bone marrow where a rare population of primitive self-renewing quiescent stem cells gives rise to expanding populations of committed progenitors that then replenish all of the blood cell lineages throughout the lifetime of the organism. When HSC divide asymmetrically, they give rise to two non-identical daughter cells, one of which is the hematopoietic progenitor cell (HPC), which in turn produces precursors with single lineage commitment, ending in terminally differentiated mature cells. Indeed, this is due to an unequal redistribution of cell-fate determinants in the cytoplasm, causing one cell to retain the stemness capacity while committing the other into multilineage differentiation. Replacement of both myeloid and lymphoid lineages is dependent upon long-term reconstituting HSC (LT-HSC), which can be isolated from the bone marrow based on their unique pattern of surface markers, defined as the CD nomenclature. The markers used in the mouse are lineage⁻, c-Kit⁺, Sca-1⁺, flk2/CD135⁻, and CD34⁻, whereas; CD34⁺, CD38⁻, CD45RA⁻, and Thy1⁺ are used in humans (Notta et al., 2011). The LT-HSC divide to produce a copy of themselves and a more differentiated cell termed as the short-term reconstituting HSC (ST-HSC). ST-HSC, recognized by the markers such as lineage⁻, c-Kit⁺, Sca-1⁺, flk2/CD135⁻, and CD34⁺, give rise to more committed multipotent progenitor (MPP) cells, which have the following markers: lineage⁻, c-Kit⁺, Sca-1⁺, flk2/CD135⁺, and CD34⁺. These last cells will in turn give rise to the multipotent common lymphoid progenitors (CLPs) that produce lymphoid lineages and to the multipotent common myeloid progenitors (CMPs) that produce myeloid lineages (Niedernhofer, 2008a). These progenitors have no self-renewal capacity; instead, they have a high proliferative potential associated to a progressive differentiation. In the lymphoid lineage, CLPs give rise to the pro-B and pro-T which are responsible for the formation of B and T lymphocyte lineages, in addition to natural killer (NK) cells. In the myeloid lineage, CMPs give rise to GMPs (Granulocyte /Macrophage progenitors) and MEPs (Megakaryocyte /Erythroid progenitors), which will differentiate into granulocytes, monocytes, platelets and erythrocytes (**Figure 3**).

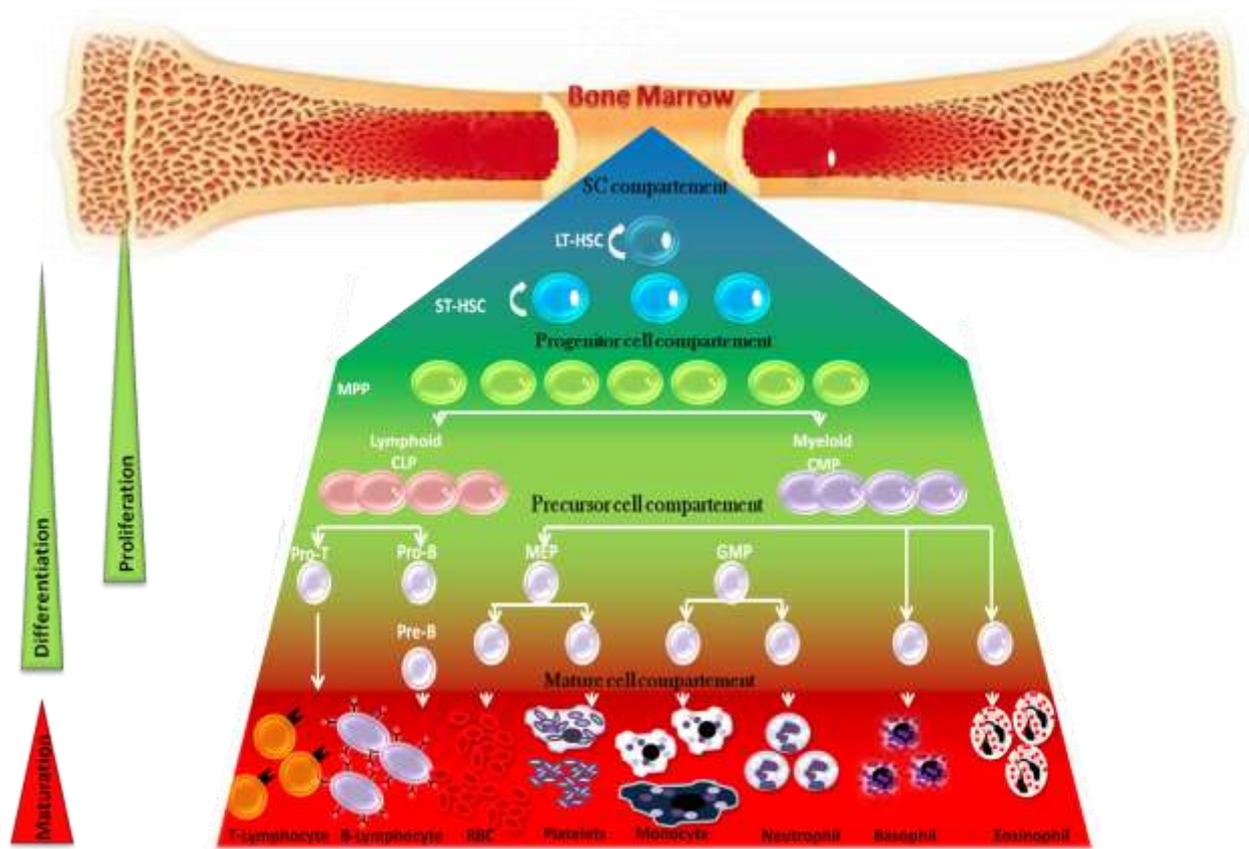


Figure 3. HSC are at the apex of the hematopoietic system. The main site for hematopoiesis is the bone marrow. The hematopoietic system consists of the stem cell (SC), the progenitor cell, the precursor cell, and the mature cell compartments. In the SC compartment, long-term HSC (LT-HSC) reside at the apex giving rise to short term HSC (ST-HSC). In the progenitor cell compartment, the multipotent progenitors (MPP) give rise to common lymphoid progenitor (CLP) and common myeloid progenitor (CMP). In the precursor cell compartment, precursor-T and -B (pre-T and -B) gives rise to T- and B-lymphocytes, megakaryocyte erythroid progenitor (MEP) gives rise to erythrocytes on red blood cells (RBC) and megakaryocytes that give platelets, granulocyte macrophage progenitor (GMP) gives rise to granulocytes (eosinophils, basophils, neutrophils) and to monocytes that will change into macrophage.

B. HSC definition

HSC are the best known and characterized ASC. They are self-renewing cells at the foundation of the entire adult blood system. Blood is one of the most highly regenerative tissues, with approximately one trillion cells arising daily in adult human bone marrow (BM). Given the fast turnover in mature blood cells, only HSC, which are by definition able to self-renew while keeping their multilineage properties, can sustain blood production during the entire life of an organism (Boisset and Robin, 2012). These HSC are functionally defined by their ability to mediate long-term repopulation of all blood lineages and their capacity to continuously reproduce lost blood cells. The multipotency property allows single HSC to differentiate and proliferate into more committed progenitors and precursors that will then produce all mature cells from the erythroid, myeloid and lymphoid lineages by a process

termed as hematopoiesis. Thus, the hematopoietic system consists of stem cells, progenitors, precursors and mature cells. This dynamic process replenishes more than 7×10^9 blood cells (leukocytes, erythrocytes and platelets) per kg body weight per day which play important roles in oxygen transport, immunological defence, and other functions. The majority of HSC reside in the bone marrow (BM), however they can be present in a variety of other tissues such as umbilical cord blood (UCB) and peripheral blood (PB) (Morrison et al., 1997). Under normal conditions, most HSC are quiescent in the G_0 phase of the cell cycle, whereas only a small fraction of cells enter the cell cycle and differentiate. On the other hand, under stress, HSC are stimulated to enter the cell cycle, proliferate and produce mature blood cells, under stress conditions (Jing et al., 2010; da Silva et al., 2010; Smith, 2003).

C. HSC discovery

Evidences for the existence of the HSC were detected by several observational and functional studies. Early anatomists have observed a wide morphological variety of cells in the bone marrow (BM) representing various blood lineages and stages of differentiation. To explain this diversity, Maximow, a Russian biologist, postulated in 1909 that hematopoiesis is organized as cellular hierarchy derived from a HSC serving as a common precursor (Maximow et al., 1909). Later, during the atomic era, existence of HSC was strongly validated by the observation that injection of radiation-exposed recipients with spleen or marrow cells from unirradiated donors allowed the rescue of these patients from death due to BM failure (Lorenz et al., 1951). However, these studies did not formally identify whether multiple stem cells restricted to each blood lineage exist, or whether a single multipotential HSC exist which warranted the need to move from observational to functional studies. In the early 60's, Till and McCulloch were the first to demonstrate the multipotentiality and self-renewal properties of HSC through series of original experiments. They demonstrated the ability of a subset of cells within the BM to form macroscopic colonies on transplantation into the spleens of lethally irradiated recipient animals (Till and McCulloch, 1961). The colonies termed colony-forming unit spleen (CFU-S), were found to contain differentiated progeny of multiple blood lineages (Till and McCulloch, 1961), and only a subset of these colonies could reform CFU-S when transplantable into secondary hosts (Siminovitch et al., 1963). Although originally believed to be derived from hematopoietic stem cells (HSC), it is noteworthy that the CFU-S described by Till and McCulloch were later found to be derived from more committed progenitor cells (Schofield, 1978), thus providing an important lesson regarding the complexity of stem and progenitor cell biology. Thus, the field of adult stem cell biology was launched by the pioneering experiment of Till and McCulloch which stimulated the

development of clonal *in vitro* assays that have culminated in today's finely detailed view of the blood system as a developmental hierarchy with multipotent HSC at the apex and terminally differentiated cells on the bottom.

D. HSC ontogeny

In the embryo, HSC production occurs in waves. The earliest haematopoiesis activity is recorded at the level of blood islands in the yolk sac, in which HSC originate from the mesoderm. Later on, a wave of production appears in the AGM region (Aorta, Gonads, mesonephros). Furthermore, another source of HSC exists in the fetus at the level of liver and placenta. These HSC arise around weeks four to six in human gestation, migrate to the liver, and then to the spleen and thymus. Near the time of birth, they migrate towards the bone marrow (BM), which becomes the unique production site throughout adult life, and start to occupy the whole BM space, just after birth. Later in childhood, the sites of blood formation become limited and occur in specific areas such as the flat bones (skull, vertebrae, thoracic cage and pelvis) and the proximal parts of the long bones of upper and lower limbs. In adults, HSC reside in a microenvironment in the BM which controls the balance between HSC maintenance and differentiation. HSC can also be found in the peripheral blood (PB) due to their migration capacity (Mikkola and Orkin, 2006; Orkin and Zon, 2008).

E. Sources of HSC

1. Umbilical cord blood

The blood, placenta and umbilical cord of the fetus are rich sources of HSC. In the late 1980s, umbilical cord blood (UCB) was recognized as an important clinical source of fetal non-embryonic multipotent HSC after birth (Barker and Wagner, 2003; Koh and Chao, 2004). These cells are easily extracted from the placenta and umbilical cord at the time of delivery, by venipuncture and gravity drainage. HSC derived from UCB having a unique potential of producing blood cells, even though, they are of wide medical importance where they are effective in treating blood diseases such as leukemia and anemia (Guilak et al., 2010; Reddy et al., 2007). Nowadays, as a mature medical service, umbilical cord blood of each new born baby is directly frozen in cord blood banks to be used in the future if needed (Navarrete and Contreras, 2009). The use of UCB as source of HSC has many advantages that include its availability, ease of harvest, and the reduced risk of graft-versus-host-disease (GVHD).

However, the use of UCB HSC imposes several limitations such as the limited number of cells that can be harvested and the delayed immune reconstitution observed following UCB transplant. Interestingly, for treatment purposes, a new strategy has evolved to create a compatible UCB donor. This strategy consisted of first producing embryos by in vitro fertilization, second implanting the embryo with transplantation antigens matching those of the affected sibling, third collecting the UCB from the resulting new born which is used to treat the sibling. However, this strategy imposes ethical problems and is under controversial debate (Grewal et al., 2004).

2. Bone Marrow

The adult BM comprises two populations of multipotent precursor cells that are the HSC and the marrow stromal cells (MSC) (Lagasse et al., 2000). In adults, the best-known location for HSC is bone marrow under steady-state conditions so that bone marrow transplantation (BMT) is directly correlated with HSC transplantation. However, large numbers of HSC are released into the blood under cytokine mobilization in a phenomenon known as blood mobilization, which opened gates for peripheral blood stem cell transplantation (PBSCT). BMT and PBSCT are the current clinical procedures to restore destroyed stem cells by high doses of chemotherapy and/or radiation therapy. Indeed, as a clinical source of HSC, mobilized peripheral blood is now replacing bone marrow as a clinical source of HSC since harvesting peripheral blood is easier for the donors than harvesting bone marrow which requires general anesthesia (Körbling and Freireich, 2011). Moreover, Ivanovic et al. has introduced new method for collection of CD34 stem cells from steady state peripheral blood (SSPB) of donors using leukodepletion filters (LDF) that are routinely discarded. The CD34 stem cells isolated from LDF exhibit unimpaired functional capacities suggesting that SSPB could become an attractive source of HSCs for hematopoietic transplantation (Ivanovic et al., 2006).

F. HSC purification

HSC are considered as a heterogeneous population containing long term and short term repopulating HSC (LT-HSC versus ST-HSC). Purification of HSC was been remarkably improved owing to the technical advances in flow cytometry and the development of monoclonal antibodies. HSC can be identified based on various antigenic markers on their

surface, lack of lineage markers and low staining with vital dyes (Mayle et al., 2013; Szilvassy and Cory, 1993).

1. Vital dyes

HSC can be phenotypically characterized by fluorescent vital dyes such as rhodamine 123 (Rho-123) (Ploemacher and Brons, 1988; Spangrude and Johnson, 1990), Hoechst 33342, Pyronin Y (Bertoncello and Williams, 2004) or to the nontoxic fluorescent substrate of ALDH (Aldefluor) (Jones et al., 1996; Krause et al., 2001). Concerning rhodamine 123 staining, its differential accumulation in mitochondrial membranes has proved to be a powerful discriminator between HSC and its progenitors. Indeed, HSC are stained less brightly than more mature progenitor cells due to their expression of higher levels of the multidrug resistance efflux pump, P-glycoprotein, which permits expelling Rh123 more rapidly. Concerning Hoechst 33342 staining, it has been used to identify replicating or quiescent cell populations due to its ability to bind DNA in live cells in addition to purification of stem cells. The concentration of Hoechst dye is lower in HSC due to their ability to efflux the dye via membrane transport pumps, which are highly active in these cells as compared to other bone marrow cell types. When Hoechst dye fluorescence is displayed at two emission wavelengths, Hoechst blue (450 nm) and Hoechst red (675 nm), the HSC are distinctly present in the side of the fluorescent profile, hence the term “side population” (SP). The high dye efflux activity of SP cells is attributed to their high expression of multidrug-resistance ABC transporters, such as transporter p-glycoprotein (Mdr1) and Abcg2.

2. CD markers

The most prevalent mode of characterization and isolation of HSC is based on their cell-surface antigenic markers. Many of these markers belong to the cluster of differentiation (CD) series. HSC do not express markers of lineage commitment and are thus called Lin⁻. Example of markers of lineage commitment in humans are CD13 and CD33 for myeloid, CD71 for erythroid, CD19 for B cells, CD61 for megakaryocytic. Example of markers of lineage commitment in mice are B220 for B cells, Mac-1 (CD11b/CD18) for monocytes, Gr-1 for Granulocytes, Ter119 for erythroid cells, Il7Ra, CD3, CD4, CD5, CD8 for T cells. Irving Weissman's group was the first to isolate mouse hematopoietic stem cells in 1988 and was also the first to work out the markers to distinguish the mouse long-term (LT-HSC) and short-term (ST-HSC) hematopoietic stem cells (self-renew-capable), and the multipotent

progenitors (MPP, low or no self-renew capability). Noteworthy, human and mice HSC are purified based on different cocktail of markers (**Figure 4-5**). As such, mouse HSC is identified as Lin^- , Sca-1^+ , c-kit^+ , $\text{CD34}^{\text{lo/-}}$, CD38^+ , $\text{Thy1.1}^{+/lo}$ while human HSC is identified as Lin^- , $\text{c-kit}^+/\text{CD117}^+$, CD34^+ , $\text{CD38}^{\text{lo/-}}$, Thy1/CD90^+ , CD59^+ . An important application for the use of CD markers to purify stem cells was the accomplishment of Notta et al. where they were able to do single cell engraftment of CD49f^+ human cell which reconstituted the hematopoietic system upon transplantation by generating long-term multilineage grafts (Notta et al., 2011). However, not all stem cells are covered by these combinations and use of surrogate markers that partial overlap with stem cells and sometime other related cells that are not stem cells. An example on non accuracy of this system is that in humans there are hematopoietic stem cells that are $\text{CD34}^-/\text{CD38}^-$ (Bhatia et al., 1998; Guo et al., 2003). Also some later studies suggested that earliest stem cells may lack c-kit on the cell surface (Weksberg et al., 2008). For human HSC use of CD133 was one step ahead as both CD34^+ and CD34^- HSC were CD133^+ .

3. SLAM code

An alternative method to better harvest of stem cells in mice uses a signature of Signaling lymphocyte activation molecule (SLAM) family of cell surface molecules that is conserved across the mouse strains. SLAM family is a group of more than 10 molecules whose genes are located mostly tandemly in a single locus on chromosome 1, all belonging to a subset of immunoglobulin gene superfamily, and originally thought to be involved in T-cell stimulation. This family includes CD48, CD150, CD244, CD229, etc. CD150 is the founding member and is also called slamF1 (SLAM family member 1). According to SLAM code, HSC are identified as $\text{CD150}^+\text{CD48}^-$ LSK, MPPs are identified as $\text{CD150}^-\text{CD48}^-$ LSK, hematopoietic progenitor cells (HPC-1) are identified as $\text{CD150}^-\text{CD48}^+$, and HPC-2 are identified as $\text{CD150}^-\text{CD48}^+$ (Kiel et al., 2005). Recently, Oguro et al has recently showed that LSK cells can be subdivided into a hierarchy of seven populations using four SLAM markers CD150, CD48, CD229 and CD244. Differences in CD229 and CD244 expression subdivided $\text{CD150}^+\text{CD48}^-$ LSK HSCs into two fractions $\text{CD229}^-\text{CD244}^-$ HSC-1 and $\text{CD229}^+\text{CD244}^-$ HSC-2, subdivided the $\text{CD150}^-\text{CD48}^-$ LSK MPPs into three fractions $\text{CD229}^-\text{CD244}^-$ MPP-1, $\text{CD229}^+\text{CD244}^-$ MPP-2 and $\text{CD229}^+\text{CD244}^+$ MPP-3 (Oguro et al., 2013).

Finally, each method has its own advantages and drawbacks. Interestingly the three different schemes for HSC purification overlap. For instance, by gating KLS $\text{CD34}^- \text{Flk2}^-$ and KLS $\text{CD150}^+ \text{CD48}^-$ cells to a Hoechst plot, it was found that 90% of those cells are SP cells. These

results suggest that different purification strategies result in very similar purified HSC populations, which should thus be largely comparable functionally and molecularly. Therefore, surface markers and metabolic fluorochromes may also be combined in addition to a combination of signaling lymphocyte activation molecule (SLAM) markers may be combined for an ideal purification of HSC (Mayle et al., 2013; Ratajczak, 2008) (**Figure 4-5**).

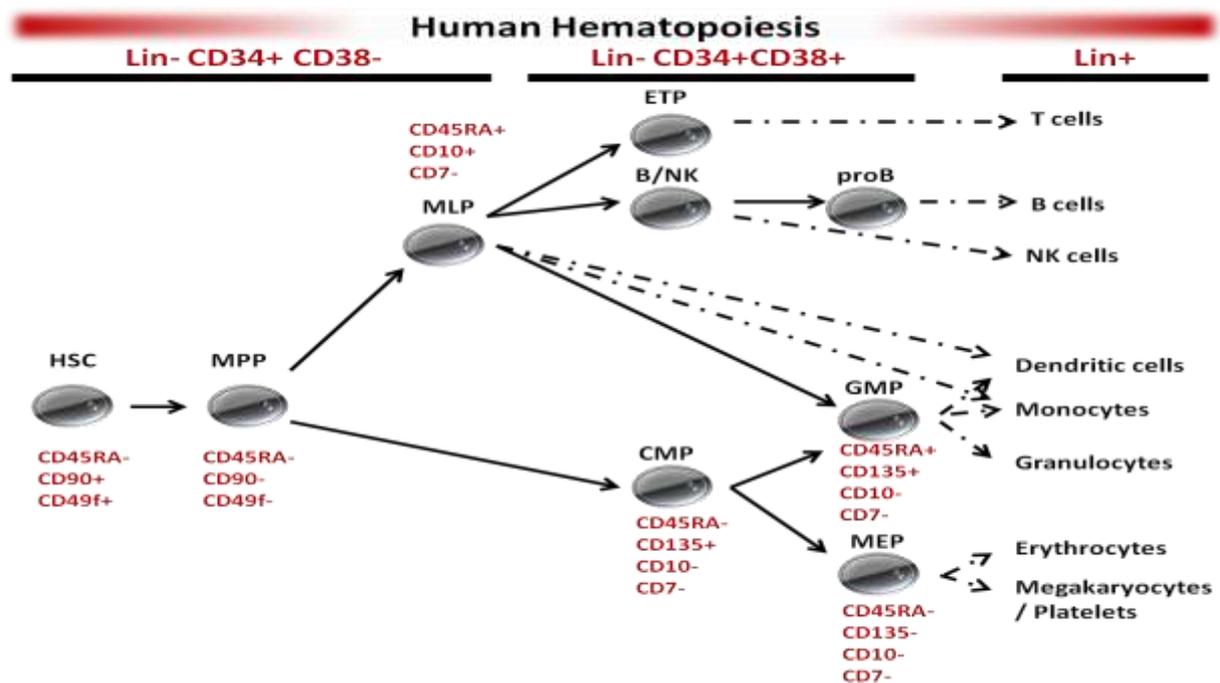


Figure 4. Current model of lineage determination in the human hematopoietic hierarchy based on phenotypic characterization. The major classes of stem and progenitor cells are defined by cell surface phenotypes listed next to each population. Terminally differentiated cells are shown on the right, and inferred lineage relationships are depicted with arrows. Human HSC are defined by the expression of CD49f and other markers, MPPs can be identified by the loss of CD49f expression; however, only one population of immature lymphoid progenitors (MLPs) has been described. In addition, mice and humans have well-defined populations of myelo-erythroid progenitors: CMPs, GMPs, and MEPs. Lin: cocktail containing cell surface markers for all terminally differentiated populations (B cell; T cell; NK; dendritic cell, monocyte, granulocyte, megakaryocyte, and erythrocyte). *Figure designed based on information from (Doulatov et al., 2012).*

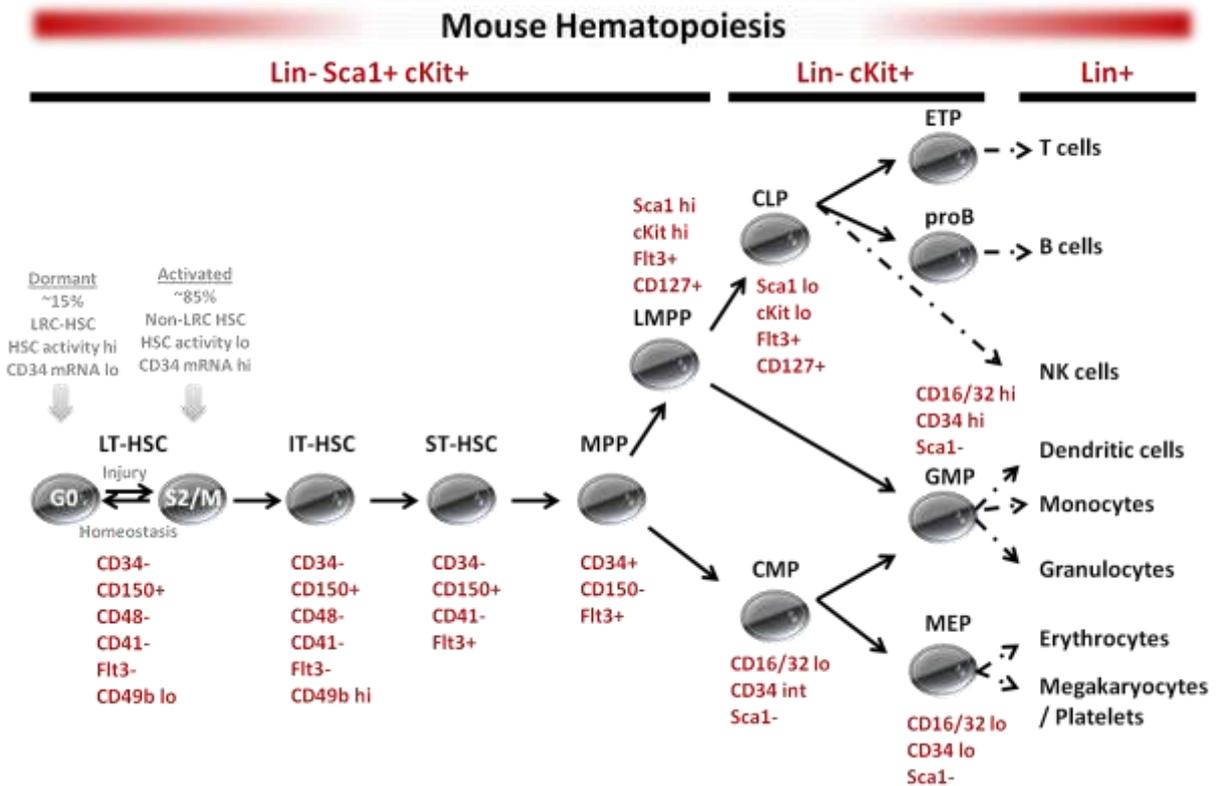


Figure 5. Current model of lineage determination in the murine hematopoietic hierarchy based on phenotypic characterization. The major classes of stem and progenitor cells are defined by cell surface phenotypes listed next to each population. Terminally differentiated cells are shown on the right, and inferred lineage relationships are depicted with arrows. Mice HSC can be separated into long-term (LT), intermediate-term (IT), and short-term (ST) classes based on the duration of repopulation. In mice, differentiation of HSC gives rise to transiently engrafting multipotent progenitors (MPP), a series of immature lymphoid-biased progenitors (such as LMPP) that undergo gradual lymphoid specification, and well-defined populations of myelo-erythroid progenitors: CMPs, GMPs, and MEPs. Lin: cocktail containing cell surface markers for all terminally differentiated populations (B cell; T cell; NK; dendritic cell, monocyte, granulocyte, megakaryocyte, and erythrocyte). *Figure designed based on information from (Doulatov et al., 2012; Seita and Weissman, 2010; Wilson et al., 2008).*

G. HSC classification systems

Lineage reconstitution kinetics examined in mice transplanted with cultured bone marrow cells or with limiting doses of bone marrow cells freshly obtained from adult mice (Cho et al., 2008; Müller-Sieburg et al., 2002; Muller-Sieburg et al., 2004) have revealed that HSC are heterogeneous populations and have provided space to classify HSC based on lineage balance using different criteria.

Muller-Sieburg et al. (Müller-Sieburg et al., 2002; Muller-Sieburg et al., 2004) classified HSC into three categories that are Myeloid-biased “My-bi”, Balanced “Bala”, and Lymphoid-biased “Ly-bi” HSC based on the proportion of lymphoid to myeloid cells (the L/M ratio) among test-donor-derived cells using in vivo limiting dilution analysis and time span of more

than 20 weeks after transplantation to assess long-term reconstitution. My-bi HSC, of L/M ratio less than 3, reconstitute the myeloid lineage after varying latencies, followed by gradual reconstitution of the lymphoid lineage. Ly-bi HSC, of L/M ratio more than 10, show both myeloid and lymphoid lineage reconstitution from early stages such that myeloid reconstitution is often detectable for only a few months but lymphoid reconstitution can persist relatively longer. Bala HSC, of L/M ratio between 3 and 10, reconstitute the lymphoid lineage soon after the myeloid lineage (Cho et al., 2008; Müller-Sieburg et al., 2002; Müller-Sieburg et al., 2004).

Eaves et al. classified HSC into three categories that are α , β , γ/δ cells based on the percentage of myeloid chimerism relative to that of lymphoid chimerism (the M/L ratio) thereby taking into account the competitor cell contribution. In this system, long-term reconstitution is assessed 16 weeks or more after transplantation. Single-donor cells are designated as α cells when the M/L ratio exceeds 2 and as β cells when the M/L ratio exceeds 0.25 but is less than 2; both α and β cells are transplantable into secondary recipient mice. Single-donor cells as designated as γ or δ cells when M/L ratio is less than 0.25 such that γ cells are designated by more than 1% myeloid chimerism exceeds whereas δ cells are designated by less than 1% myeloid chimerism; both γ and δ cells are not transplantable into secondary mice (Benz et al., 2012; Dykstra et al., 2007).

Ema et al. proposed classification of HSC into three categories that are long term (LT-), intermediate term (IT-), and short term (ST-) HSC based on reconstitution time period (Yamamoto et al., 2013) of granulocytes that directly reflects HSC activity (Dykstra et al., 2007; Jordan and Lemischka, 1990). Reconstitution time period for ST-HSC is less than 6 months, for IT-HSC is less than 12 months and for LT-HSC is greater than 12 months in mice. After secondary transplantation, ST-HSC do not show any reconstitution activity, IT-HSC reconstitution ability begin to decrease by 6 months whereas LT-HSC reconstitution levels do not change.

Interestingly, Ema et al. reported correspondence in the three classification systems by using data of transplantation with 30 single HSC (Morita et al., 2010). Therefore, α , β , and γ/δ cells likely correspond to My-bi HSC, Bala HSC, and Ly-bi HSC, respectively. Also she reported that LT-HSC overlap with myeloid-biased HSC or α cells, IT-HSC exhibit varying reconstitution patterns, ST-HSC overlap with lymphoid-biased HSC suggesting that HSC lifespan is linked to cell differentiation.

H. HSC differentiation models

Weissman's group has proposed a bifurcation model (Akashi et al., 2000) in which a HSC, after loss of self renewal, give rise to multipotent progenitors (MPPs), that maintains all differentiation potentials, which in turn give rise to common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs) in adult bone marrow (Akashi et al., 2000; Kondo et al., 1997) and fetal liver (Mebius et al., 2001; Traver et al., 2001). CLPs and CMPs are mutually exclusive populations where CLPs give rise solely to B cells, T cells, and natural killer (NK) cells whereas CMPs give rise solely to granulocytes, macrophages, erythrocytes, and platelets. However, MPPs have not been identified at the clonal level until this date and the relationship of MPPs to CLPs or CMPs is yet to be clarified.

Katsura 's group has proposed a myeloid-based model in which HSC give rise to myeloid erythroid (MyE) or granulocyte/macrophage/B cell/T cell (MyBT) progenitors and MyBT progenitors then give rise to either MyB or MyT progenitors (Katsura and Kawamoto, 2001; Lu et al., 2002). In fact the model was based on their successful detection of MyB progenitors and MyT progenitors (Kawamoto et al., 1997, 1999; Lu et al., 2002) and inability to detect BT progenitors. However, the drawbacks of this model is that they did not distinguish between HSC and MPP due to usage of an in vitro assay known as a multilineage progenitor assay (Kawamoto et al., 1997) to analyze fetal liver cells (Jenkinson et al., 1992) that was not an efficient technique to detect HSC. In addition, in this proposed system, they did not examine megakaryocytes and they did not compare progenitors in adult bone marrow with those in fetal liver.

Jacobsen's group has proposed LMPP model which combined elements of the bifurcation and myeloid-based models (Adolfsson et al., 2005). In this model, HSC give rise to megakaryocyte/erythrocyte progenitors and lymphoid-primed MPPs (LMPPs) that give rise to granulocyte/macrophage progenitors and CLPs that represent B/T cell lineages but not the megakaryocyte/erythrocyte lineage (Adolfsson et al., 2005; Månsson et al., 2007). This model was based on using in vitro clonal assays that enabled detection of myeloid potential of single LMPPs and coculture with OP9 and OP9/Delta-like 1 stromal cells plus cytokines to detect B and T cell potentials respectively. From these analyses, the authors proposed a model combining elements of the bifurcation and myeloid-based models (Adolfsson et al., 2005).

Recently, this year, Suda's group has proposed a myeloid bypass model for HSC differentiation. In this model, LT-HSC give rise to either rCMPs, also referred to as megakaryocyte progenitors with repopulating potential, or IT- and ST-HSC that in turn give

rise to B or T cell progenitors with myeloid potential (MyB and MyT progenitors). The model suggests that, in comparison to lymphoid compartment, the myeloid compartment is established earlier and is larger in the bone marrow and the B lymphoid compartment is larger than the T lymphoid compartment which is consistent with the fact that bone marrow is the site of myelopoiesis and B lymphopoiesis but not of T lymphopoiesis. The model was based on performing paired daughter cell (PDC) assay (Ema et al., 2000; Suda et al., 1984; Takano et al., 2004) on one single HSC in which the daughter cell it produce is separated by a micromanipulator and transplanted with competitor cells leading to emergence of pairs of LT- or ST-HSC and megakaryocyte progenitors, as well as pairs of ST-HSC and CMPs with repopulating potential (rCMPs) suggesting that cells of the myeloid or megakaryocyte lineage are generated directly from HSC via asymmetric division. However, this model does not provide clear information on lymphoid differentiation pathways because CLP cells are extremely rare in bone marrow (Inlay et al., 2009) and have never been detected in single-cell transplantation (Morita et al., 2010; Yamamoto et al., 2013) (**Figure 6**).

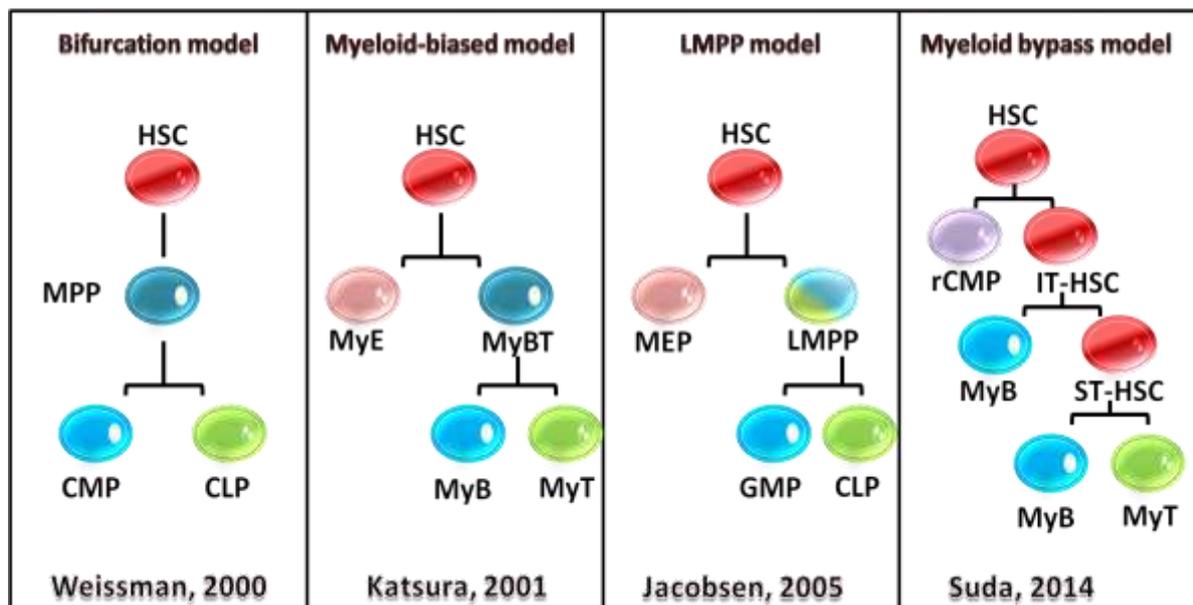


Figure 6. HSC differentiation models. HSC differentiation models shown are the bifurcation model, myeloid-based model, LMPP model and the recent myeloid bypass model. IT-HSC, intermediate term-HSC; ST-HSC, short term-HSC; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; rCMP, repopulating CMP; GMP, granulocyte and macrophage progenitor; LMPP, lymphoid-primed multipotent progenitor; MEP, megakaryocyte and erythrocyte progenitor; MPP, multipotent progenitor; MyB, myeloid progenitor with B cell potential; MyBT, myeloid progenitor with B cell and T cell potential; MyE, myeloid progenitor with erythroid potential; MyT, myeloid progenitor with T cell potential. *Figure designed based on information from (Ema et al., 2014).*

I. HSC microenvironment

HSC are located in the BM in which they interact within a specific physical microenvironment called the hematopoietic niche. This niche regulates HSC fate in terms of maintenance, quiescence, self-renewal and differentiation. HSC reside in two different niches in the BM, either the endosteal niche or the endothelium niche, also called osteoblastic niche or vascular niche; respectively. The endosteal niche is further divided into two niches: the quiescent and the self-renewing endosteal niches. In the quiescent endosteal niche, HSC reside in close contact with the endosteal surface of the bone and cells of the BM stroma (osteoblasts, osteoclasts, stromal cells and MSC). The main supportive cell type, the osteoprogenitor population, precisely Spindle-shaped N-cadherin-expressing osteoblasts (SNOs), maintains HSC in a quiescent/slow-cycling state (Calvi et al., 2003; Lo Celso et al., 2009; Raaijmakers et al., 2010; Zhang et al., 2003). In response to injury, quiescent HSC might be activated and recruited to the vascular niche, which promotes differentiation and expansion along megakaryocytic and other myeloid lineages. These HSC in the vascular niche are in direct contact with endothelial cells lining the sinusoids (Jing et al., 2010; Reddy et al., 2007). The self-renewing endosteal niche would contain quiescent HSC mixed with dividing HSC. Self-renewing HSC produce multipotential progenitors (MPPs) either by divisional or environmental asymmetry. In turn, MPPs can give rise to all hematopoietic lineages. In turn, MPPs can give rise to all hematopoietic lineages, including B-cell and T-cell precursors. Indeed, B-cell precursors constitute the B cell niche whereas T-cell precursors would later migrate to the thymus where they enter a microenvironment, promoting T-cell maturation (Wilson and Trumpp, 2006).

Recent data suggest that quiescent and self-renewing HSC are primarily located in the endosteal niche whereas dividing and differentiating HSC reside in the vascular niche, where they can be released into the circulation. Moreover, the niche comprises nerve cells which play a key role in HSC regulation (Jing et al., 2010). More recently, data has also shown that mature hematopoietic cells such as macrophages (Orkin and Zon, 2008) and megakaryocytes (Bradley and Metcalf, 1966) are also active participants in the hematopoietic niche, where they play an important role in the HSC maintenance.

This implies that HSC are not autonomous units of development; rather, tissue specific niches control their destiny. In their microenvironment, HSC are quiescent and have a low rate of proliferation capacity that results in a limited number of divisions. This will result in an increase in their resistance against stresses such as radiations, chemical agents, reactive oxygen species (ROS), and mutations. This is important for the long-term maintenance of

stem cells. In the niche, stromal cells modulate hematopoietic development through complex mechanisms involving growth factor production as well as cell-cell interactions (Bartek and Lukas, 2007; Pang et al., 2011). Finally, the interaction of HSC with their particular niches is critical for maintaining stem cell properties, including cell adhesion, survival, and cell division (Yamazaki et al., 2011). Oxygen also plays a physiological role in the regulation of HSC function. In fact, bone marrow is a healthy tissue that represents an oxygen gradient that regulates HSC distribution and function, with regions of hypoxia. Using Hoechst dye, a recent study showed that BM HSC are organized according to regional blood supply and oxygen levels *in vivo*. This methodology allows correlates the intensity of Hoechst staining with the degree of oxygenation (Chow et al., 2011) (**Figure 7**).

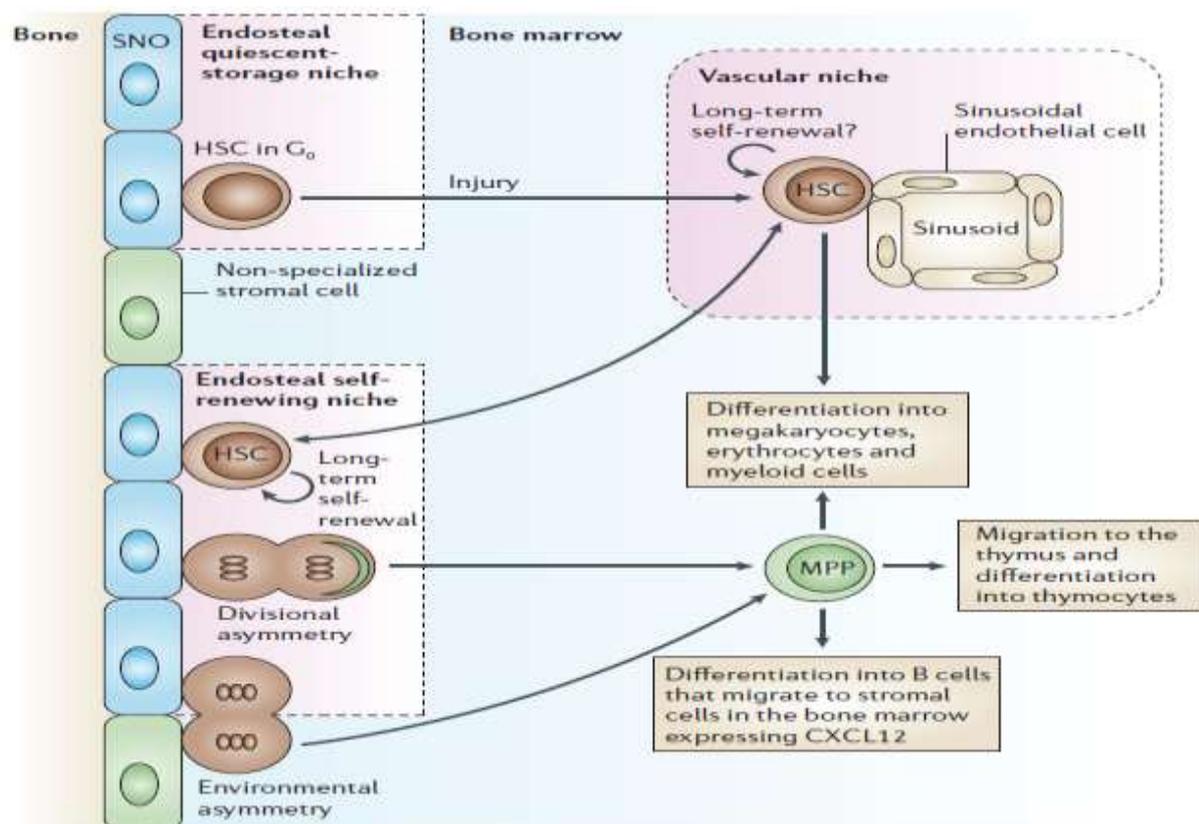


Figure 7. Model of BM-HSC niche. Endosteal bone surfaces are lined with stromal cells. Spindle-shaped N-cadherin-expressing osteoblasts (SNOs) serve as niche cells to maintain quiescence and prevent differentiation of attached hematopoietic stem cells (HSC). The quiescent endosteal niche would maintain dormant HSC long-term. In response to injury, quiescent HSC might be activated and recruited to the vascular niche. The self-renewing niche would contain quiescent HSC intermingled with dividing HSC. Self-renewing HSC produce multipotential progenitors (MPPs) either by divisional or environmental asymmetry. More HSC can be generated by symmetrical divisions which might provide the vascular niche with new HSC. Whether HSC long-term self-renew in the vascular niche remains to be determined, and it is probable that influx of HSC from endosteal niches is necessary to ensure prolonged hematopoietic-cell production at the vascular niche. HSC in the vascular niche promote differentiation and expansion along megakaryocytic and other myeloid-cell lineages, particularly in response to injury. MPPs can give rise to all hematopoietic lineages, including B-cell precursors attached to randomly distributed CXC-chemokine ligand 12 (CXCL12)-expressing stromal cells that constitute a B-cell niche. Unidentified T-cell precursors migrate to the thymus where they enter a microenvironment, promoting T-cell maturation. *Figure adapted from* (Wilson and Trumpp, 2006).

J. HSC bioenergetics

Dormancy or long-term quiescence is likely a fundamental attribute of adult tissue stem cells, such as HSC and hair follicle stem cells, which renders them able to avoid damage accumulation from physiological stress (Fuchs and Horsley, 2011).

1. Quiescence of HSC

In both mouse and human, infrequent cycling of HSC appears to be a protective mechanism against accumulation of DNA damage due to oxidative and replicative stress. In mice, label-retaining cell studies estimated that LT-HSC divide once every 30–50 days (Cheshier et al., 1999; Kiel et al., 2007). Other studies showed the existence of even more dormant subpopulation of LT-HSC representing only ~15% of the phenotypic LT-HSC pool that only divides about five times in a mouse's lifetime (Foudi et al., 2009; Wilson et al., 2008) and that are thought not to contribute to daily production of hematopoietic cells, but to serve as a reservoir in case of injury. In humans, studies based on investigation of mean telomere length (Shepherd et al., 2004) and X chromosome inactivation ratios (Catlin et al., 2011), estimated that human HSC divide every 175–350 days. In addition, cell cycle analyze revealed that the majority of HSC are in G₀.

a. Hypoxic microenvironment

First evidence supporting the existence of quiescent HSC is that they reside in hypoxic microenvironment in the bone marrow. For instance, it has been demonstrated that LT-HSC reside within the endosteal zone of the bone marrow (Arai et al., 2004; Calvi et al., 2003; Zhang et al., 2003) which is scarcely perfused with blood capillaries and which possess very low partial oxygen pressure (Draenert and Draenert, 1980). In addition, the BM is densely populated with blood cells that consumes massively oxygen so that oxygen pressure was found to decrease by 10-fold at a distance of several cells from the nearest capillary (Chow et al., 2001). Moreover, human cord blood HSC transplanted into super-immunodeficient NOD/scid/IL-2R γ (NOG) mice reacquired both hypoxic status and cell cycle quiescence after transplantation (Shima et al., 2010). In vitro data also showed that function of HSC is maintained most effectively under hypoxic conditions as for example culturing of BM cells in

hypoxia elevates colony-forming ability or transplantation capacity (Cipolleschi et al., 1993; Danet et al., 2003; Ivanovic et al., 2004).

b. Hypoxic-responsive regulatory pathway

The quiescent state of HSC has been also proved at the molecular level where HSC exhibit hypoxia-responsive regulatory pathways among which hypoxia-inducible factor-1 (HIF-1) is a key player (Semenza, 2010) being validated to be expressed at transcriptional and proteomic level in LT-HSC (Simsek et al., 2010; Takubo et al., 2010). In brief, HIF-1 is a heterodimeric protein consisting of the O₂-regulated HIF-1 α subunit and a constitutively expressed HIF-1 β subunit (Wang and Semenza, 1995). Under normoxic conditions, HIF-1 α is hydroxylated at proline (Kaelin Jr. and Ratcliffe, 2008) by three prolyl hydroxylases (PHD1–3) (Epstein et al., 2001) which is then recognized by the von Hippel-Lindau (VHL) tumor suppressor protein, which recruits the Elongin C/Elongin B/Cullin2/E3-ubiquitin-ligase complex, leading to ubiquitinylation and subsequent proteasomal degradation. Under hypoxic conditions, HIF-1 α prolyl hydroxylation is suppressed, stabilizing HIF-1 α protein (Kaelin Jr. and Ratcliffe, 2008) that translocates to nucleus to bind HIF-1 β forming a heterodimer that will bind to hypoxia response elements (HREs) in numerous target genes and activate transcription (Semenza, 2010). Therefore, several studies showed that HIF-1 α plays an essential role in the maintenance of HSC quiescence and stress resistance. Using HIF-1 α knockout mice showed loss of HSC capacity for marrow reconstitution during serial BM transplantation (Takubo et al., 2010), drove LT-HSC into cell cycle and reduced tolerance to stresses such as 5-fluorouracil administration or aging. In addition, several evidence indicate that HIF-1 is implicated in reprogramming glucose metabolism in hypoxic cells by mediating an active switch from oxidative to glycolytic metabolism (Luo et al., 2011; Semenza, 2009; Wheaton and Chandel, 2011). Also, under hypoxic conditions, cells increase flux through the glycolytic pathway via HIF-1-dependent transcriptional activation of genes encoding glucose transporters, glycolytic enzymes, and metabolic regulatory enzymes (Iyer et al., 1998). Taken together, these findings indicate that HIF-1 orchestrates molecular responses that maintain redox homeostasis in the face of changing O₂ levels. Thus, cells reduce their ROS production under conditions of chronic hypoxia, but only if HIF-1 is active.

2. Metabolic shift in HSC upon differentiation

Stem cells, in general, have been reported to be quiescent whereas their progenitors are actively cycling. In this context HSC exhibit few mitochondria whereas progenitor cells contain many mitochondria. Thus, the transition from stem to progenitor state is accompanied by metabolic change from anaerobic glycolysis to oxidative phosphorylation. HSC, in particular, have been also reported to be quiescent whereas progenitors and mature cells are more cycling. For instance, among LSK cells (Lineage⁻ Sca-1⁺ c-Kit⁺), that encompasses both HSC and their progenitors, CD34⁻, CD150^{hi}, and side population (SP) cells are slow cell cycling (Goodell et al., 1996; Kiel et al., 2005; Osawa et al., 1996). Furthermore, Wilson et al. showed that more than 70% of CD34⁻CD48⁻CD150^{hi}LSK HSC are in G₀ and out of this population ~30% is considered dormant dividing only every 145-193 days and ~70% is considered homeostatic or activated population dividing every 28–36 days. Moreover, in the same study they showed that less than 10% of MPP cells identified as CD34⁺ LSK cells are quiescent (Wilson et al., 2008). Similarly, Takizawa et al. showed that in steady state murine bone marrow both fast-cycling cells known to undergo four to seven divisions in 7 weeks and quiescent cells known to undergo no divisions in 12–14 weeks are present (Takizawa et al., 2011). Interestingly, cell cycle status of HSC plays fundamental role in their reconstitution potential. For example, slow cycling HSC possess long-term (LT) reconstitution activity when they are transplanted into lethally irradiated mice knowing that quiescence provides them protection from DNA damage although it can still accumulate over time due to their long lifespan and renders them resistant to cytotoxic agents. Another example, actively cycling stem cells and progenitors exhibit only short-term (ST) reconstitution, and cannot maintain hematopoiesis for more than 3–4 months knowing that active cell cycle permits accumulation of damage and their elimination by cell cycle checkpoints and apoptosis.

3. Redox signaling in HSC

a. ROS production

Redox signaling in HSC is greatly important being implicated in either physiological or pathological control for maintenance of self-renewal or induction of commitment (Boonstra and Post, 2004; Noble et al., 2005; Sauer et al., 2001). HSC bioenergetic metabolism is

accompanied by the generation of ROS (Balaban et al., 2005). ROS is a collective term for various oxygen species that are more reactive than dioxygen such as superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. Under normal physiological conditions, ROS can be formed during numerous reactions *in vivo*, including enzymatic activity, activated phagocytic cells and mitochondrial respiration and by NADPH oxidase. These reactions generate ROS as part of their normal activity. However, when ROS production exceeds the capacity of antioxidant systems to control ROS levels, oxidative stress occurs where high doses of ROS can oxidize nucleic acids, lipids, proteins, or carbohydrates, and excessive ROS can promote cellular senescence, apoptosis, or carcinogenesis (Balaban et al., 2005; Harman, 1956) (**Figure 8**).

Emerging evidence show that oxidative stress, in particular ROS content, influences stem cell migration, development, and self-renewal as well as their cell cycle status. Though high levels of ROS may harm cells by inducing DNA damage and promoting apoptosis, moderate levels of ROS are needed for hematopoiesis during embryonic development, and are also required in adult hematopoietic homeostasis. In quiescent stem cells ROS levels are kept low, thus supporting their long term repopulation ability. Elevation in ROS content drives stem cell differentiation to short-term repopulating cells and further on to myeloid differentiation.

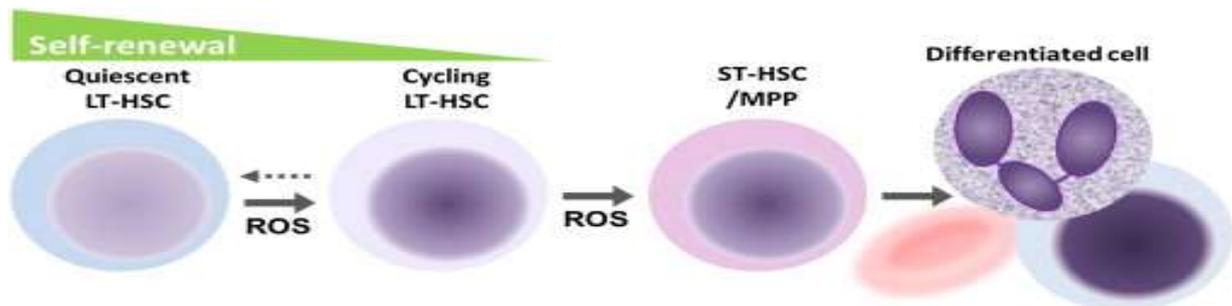


Figure 8. ROS production is increased with HSC differentiation. The long-term HSC (LT-HSC) are at the apex of the hematopoietic hierarchy and are classified into quiescent and cycling whereby ROS level is increased. LT-HSC differentiation into short-term HSC (ST-HSC), multipotent progenitors and differentiated cell is associated with increased ROS production. *Figure adapted from* (Suda et al., 2011).

Taken together, we may suggest that the quiescent and cycling state of hematopoietic stem cells involves fluctuations in ROS levels, affecting their motility, proliferation, differentiation and repopulation potential. While exceedingly high ROS levels were shown to promote exhaustion of the stem cell pool in the bone marrow, extremely low ROS levels in

hematopoietic stem cells, in models where ROS content was reduced below normal levels, showed defects in their differentiation ability, leading to their impaired repopulation capacity. Thus, minimal ROS levels are crucial for stem cell function and balanced hematopoiesis (Ludin et al., 2014).

The interaction between HSC/ HSPC and their niche has also been reported to be regulated by ROS. Indeed, quiescent stem cells in a hypoxic niche exhibit low ROS levels due to well-organized antioxidant defense systems, which protect stem cells from extrinsic oxidative stress, whereas high levels of ROS promote the differentiation or migration of stem/progenitor cells (Ushio-Fukai and Rehman, 2014).

b. NADPH oxidase (NOX) generation of ROS

The main intracellular sources of ROS encompass the mitochondrial respiratory chain complexes and a number of oxidases among which the plasma membrane NOX is recognized to be one of the major players (Finkel, 2003). Unlike the mitochondrial oxidoreductases, which generate ROS as a consequence of accidental electron leaks (Nohl et al., 2005), NOX “deliberately” catalyzes generation of O₂⁻ by single electron transfer from NADPH to O₂ therefore implying a specialized function (Babior, 1999; Cross and Segal, 2004). Besides the best-characterized neutrophil NOX2 isoform (Cross and Segal, 2004) which is involved in the bactericide “respiratory burst” other NOX isoforms in non-phagocytic cells have been discovered and their ROS generation suggested to play a role as messengers in the activation of specific signaling pathways (Bokoch and Knaus, 2003; Kamata and Hirata, 1999). In many cell types, the major ROS producer is the cell membrane-bound NAD(P)H oxidase (NOX) (Babior, 1999). NOX catalyzes the oxidation of NAD(P)H and the monoelectronic reduction of O₂ to the superoxide anion radical which is then converted by chemical or enzymatic dismutation in H₂O₂ (Babior, 2004; Cross and Segal, 2004), which is used by myeloperoxidase to oxidize chloride anions to hypochlorite. NOX encompasses two membrane-embedded subunits, p22 and the catalytic unit gp91 (Cross and Segal, 2004). The gp91/p22 heterodimer is catalytically dormant and to be activated requires the recruitment of a number of additional cytoplasmic regulatory subunits such as p40, p47 and p67 (Babior, 2004; Cross and Segal, 2004; Quinn and Gauss, 2004) which is mediated by a multitude of intracellular signaling pathways leading to phosphorylation of p47 and p67 and mediated by the activation of the small G-protein Rac2. The NOX family encompasses five isoforms, NOX-1 to -5, with NOX 1-4 more closely related and NOX2 being the earlier discovered neutrophil isoform where it is constitutively expressed (Geiszt and Leto, 2004; Quinn and

Gauss, 2004). First NOX was discovered in neutrophils and later in macrophages where it plays a major role in the host defense mechanism against bacteria by oxidative burst (Babior, 2004; Cross and Segal, 2004). Then NOX have been discovered in a number of cell types not involved in host defense (Bokoch and Knaus, 2003; Cox et al., 1987; Geiszt and Leto, 2004; Quinn and Gauss, 2004) (Figure 9).

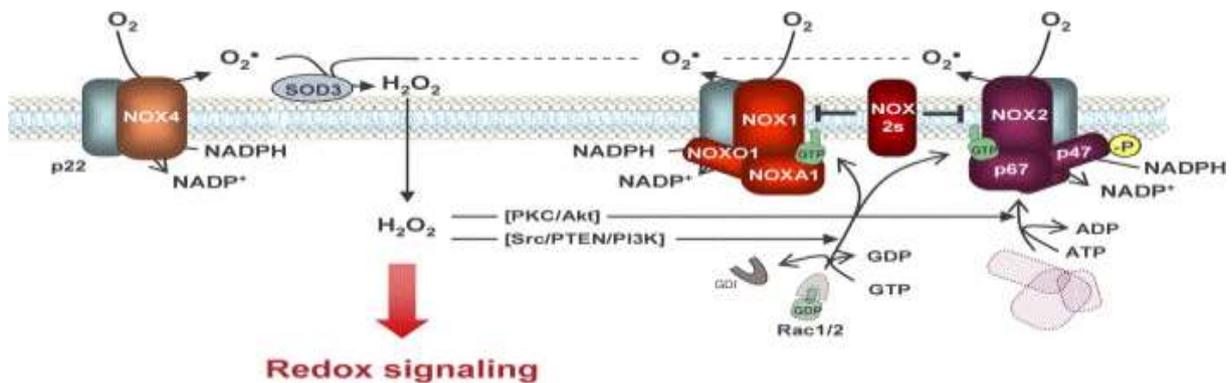


Figure 9. Schematic representation of NOX-related ROS-induced ROS production back-forward mechanism in HSC. Figure adapted from (Piccoli et al., 2007).

c. Characterization of NOX in human HSC

The study of Piccoli et al. in 2005 have revealed for the first time the occurrence of NOX activity in human CD34⁺ HSC by characterizing mitochondrial and extra-mitochondrial oxygen consuming reactions. The HSC were collected by apheresis from humans following pre-conditioning by granulocyte colony-stimulating factor and isolated by anti-CD34 positive immunoselection. Human HSC showed a low mitochondrial oxygen consumption rate and a low amount of mitochondrial respiratory chain complexes reflecting that HSC exhibit poor oxidative phosphorylation. In addition, the intracellular content of mitochondria was not homogeneously distributed in the CD34⁺ hematopoietic stem cell sample displaying a clear inverse correlation of their density with the expression of the CD34 commitment marker suggesting that differentiation of HSC is accompanied by acquisition of a more efficient energy supply system than glycolysis which depends on the mitochondrial oxidative phosphorylation apparatus. About half of the endogenous oxygen consumption was extra-mitochondrial revealing presence of extra-mitochondrial oxygen consumption which is contributed to NOX2 and NOX4 isoforms of the O₂⁻ producer plasma membrane NAD(P)H oxidase with low constitutive activity. A model is proposed suggesting for the NAD(P)H

oxidase a role of O₂ sensor and/or ROS source serving as redox messengers in the activation of intracellular signaling pathways leading to mitochondriogenesis, cell survival, and differentiation in hematopoietic stem cells. At the transcriptional level, the occurrence of a NOX-like enzyme in CD34⁺ HSC was verified by a reverse transcriptase-PCR amplification experiment carried out on whole RNA extracts from HSC samples. It was shown that only NOX2 and NOX4 were expressed in the stem cell samples along with the regulatory subunits. At translational level, to verify the presence of NOX gene products, immunofluorescent cytochemical analysis on the HSC with antibodies against the catalytic NOX2 gp91^{phox} and the regulatory p47 subunits were carried on. Noteworthy, both the fluorescent signals were clustered in a polarized manner and more important, the superimposition of the two fluorescence signals indicated large co-localization. This result clearly indicated the presence in the CD34⁺ HSC of a significant amount of NOX subunits and that possibly these were assembled in a functional complex. To support and complement the NOX subunits immuno-co-localization evidence, a set of immunoprecipitation experiments were carried out on HSC lysate showing that practically most, if not all, the CD34⁺ HSC content of NOX resulted in association of the catalytic and one of the regulatory activating subunits. It providing conclusive evidence that the HSC NADPH oxidase complex is fully assembled (Piccoli et al., 2005).

In 2007, study by Piccoli et al. highlighted the importance of redox signaling in poising the balance between self-renewal and differentiation in adult stem cells where they showed that human hematopoietic stem/progenitor cells HSPCs derived from bone marrow express multiple isoforms of NOX and produce constitutively ROS (Piccoli et al., 2007). In particular, the study showed that HSPCs constitutively generate low levels of hydrogen peroxide now to be product of NOX activity whose production is inhibited by antioxidants. Moreover, HSC expressed at the mRNA and protein levels the catalytic subunits of NOX1, NOX2, and NOX4 isoforms of along with the regulatory subunits p22, p40, p47, p67, rac1, rac2, NOXO1, and NOXA1 as well as the splicing variant NOX2s. Interestingly, co-immunostaining for the CD34 marker and one or the other of the three NOX isoforms showed that the three NOX isoforms are largely co-expressed in the same HSC (66%). It can be noticed a slight positive correlation between the expression of the uncommitment stem cell-marker CD34 and all the three NOX isoforms. More importantly the superimposition of the density contours and of the fluorescence distribution curves showed that a large part (64% ± 3, n = 3) of the CD34⁺ HSC co-expressed NOX1, NOX2, and NOX4. In addition, HSC expressed intracellular antioxidants known to be ROS-scavenging enzymes such as catalase, the mitochondrial and

cytosolic isoforms of glutathione peroxidase (Gpx) and superoxide dismutase (SOD) as well as the extracellular SOD3 isoform (Folz and Crapo, 1994) (**Figure 10**).

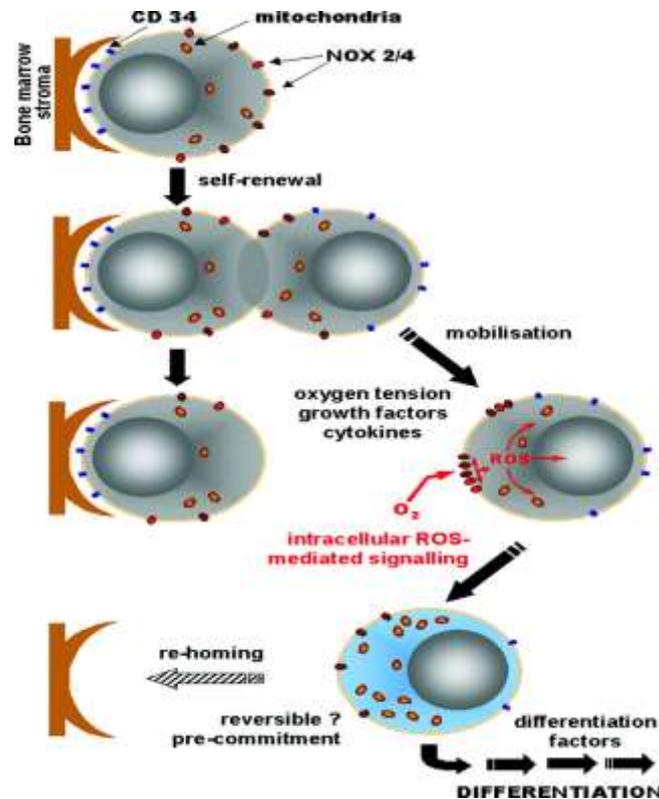


Figure 10. Function of NADPH oxidase driving ROS production in growth and differentiation of CD34⁺ HSC. Differentiation of CD34⁺ cells is accompanied by increased in number of mitochondria, increase in ROS production by NADPH oxidase, and metabolic shift away from glycolysis. *Figure adapted from (Piccoli et al., 2005).*

4. Oxidative stress in DNA damage repair

ROS plays an essential role in the DDR. ATM-deficient mice illustrate a major clue for implication of oxidative stress in DDR. ATM represents the serine/threonine kinases ataxia telangiectasia mutated (ATM) that belong to the phosphoinositide-3-like kinase kinase family (Shiloh, 2003) and is activated by double strand breaks (DSB) where it process DNA damage, maintain genome integrity and function in telomere maintenance (Herbig et al., 2004). Interestingly, *Atm* KO mice show premature senescence in stem and progenitor cells in various organs (Nitta et al., 2011; Takubo et al., 2008). *Atm* KO mice also show a defect in HSC self-renewal and exhibit a progressive decline in HSC with age (Ito et al., 2004a) that can be rescued by *p16^{Ink4a}* downregulation or suppression and more importantly can be fixed

by administration of antioxidant N-acetyl L-cysteine is administered to *Atm* KO mice, indicating a causative role for ROS in impaired function of *Atm*-deficient HSC. Moreover, ATM is reportedly a critical sensor of oxidative stress (Guo et al., 2010). These findings indicate that ATM regulates HSC self-renewal by inhibiting ROS production and p16^{Ink4a} expression to prevent HSC from undergoing premature senescence.

III. Aging of HSC

Aging is the process of system deterioration over time in the whole body. It is believed to be initiated at the embryonic stage in which some loss of cell potential is detected after the first zygotic cell division. Aging was first detected in mammalian somatic cells that upon aging had limited number of intrinsic divisions (Hayflick, 1976). Later, ontogeny-related studies suggested that even the self-renewing stem cells are not exempted from the aging process and the degree of aging is affected by intrinsic and extrinsic multifactorial conditions. HSC, known as the best-studied adult stem cells, are subjected to aging during its life-long production of blood cells. The aging of the HSC often leads to perturbations in its function to sustain the HSC pool and to maintain homeostasis.

A. HSC aging features

From a developmental perspective, hematopoietic stem cell aging can be viewed as a process that starts in embryonic development, and can be characterized molecularly and functionally by telomere shortening and reduced HSC proliferative potential, respectively. Shortening of the telomere is highly correlated with aging in HSC. For instance, researchers found that telomere length of donor bone marrow cells decreased by ~7 kb after just two rounds of transplantation and cellular division capacity was reduced with serial transplantation (Allsopp et al., 2001). In addition, HSC proliferative potential is highly correlated with aging. The stem cell pool expands rapidly after birth and then becomes relatively quiescent around a steady state (Bowie et al., 2006). For instance, the proliferative potential and ability of primitive hematopoietic cells to produce CD34⁺ progenitor cells has also been shown to decrease with the age of the cell donor (Lansdorp et al., 1993). HSC pool size in mice is larger in B6 mice that represent long-lived strains than the short lived DBA strain (de Haan et al., 1997). However, telomere length is also correlated with the replicative capacity of HSC. Previous study showed that telomere lengths in human bone marrow stem cells with the CD34⁺CD38^{lo} phenotype were found to be much shorter than those in cells from fetal liver or cord blood, but all cells showed a proliferation-associated loss of telomeric DNA (Vaziri et al., 1994). Other ex vivo expansion trials designed for clinical applications have also experienced proliferation-associated loss of telomeric DNA and cellular senescence (Engelhardt et al., 1997).

B. HSC aging consequences

Age-related changes have been reported to occur in both the mouse and recently the human hematopoietic stem cell compartment at the cell intrinsic functional and molecular level. In mouse, profound perturbations of the homeostatic control underlie aging of LT-HSC. LT-HSC aging is accompanied by cell autonomous changes, including increased stem cell self-renewal, altered lineage potential, and differential capacity to generate committed myeloid and lymphoid progenitors, and diminished lymphoid potential. Expression profiling revealed that LT-HSC aging was accompanied by the systemic down-regulation of genes mediating lymphoid specification and function and up-regulation of genes involved in specifying myeloid fate and function. Moreover, LT-HSCs from old mice expressed elevated levels of many genes involved in leukemic transformation (Rossi et al., 2005). In humans, HSPC undergo quantitative and functional modifications with age. In the BM of elderly (>70 years) individuals, the proportion of multipotent cells (CD34⁺CD38⁻) increases, the frequency of myeloid progenitors persist at the same level, while the frequency of early and committed B-lymphoid progenitors decreases with age. In addition, the frequency of NSG repopulating cells does not change significantly with age during transplantation and there is a decrease in myeloid lineage reconstitution which was seen also in colony-forming assays *in-vitro* (Kuranda et al., 2011).

Therefore, HSC aging phenomenon reflects an increase in number of HSC is counterbalanced with decrease in their proliferative potential. In addition, HSC aging is accompanied by diminished self-renewal capacity, a decrease in differentiation and engraftment potential, and most importantly a lymphoid/myeloid lineage imbalance that results in immune dysfunction, immunosenescence, and increasing prevalence of myeloproliferative disease (**Figure 11**).

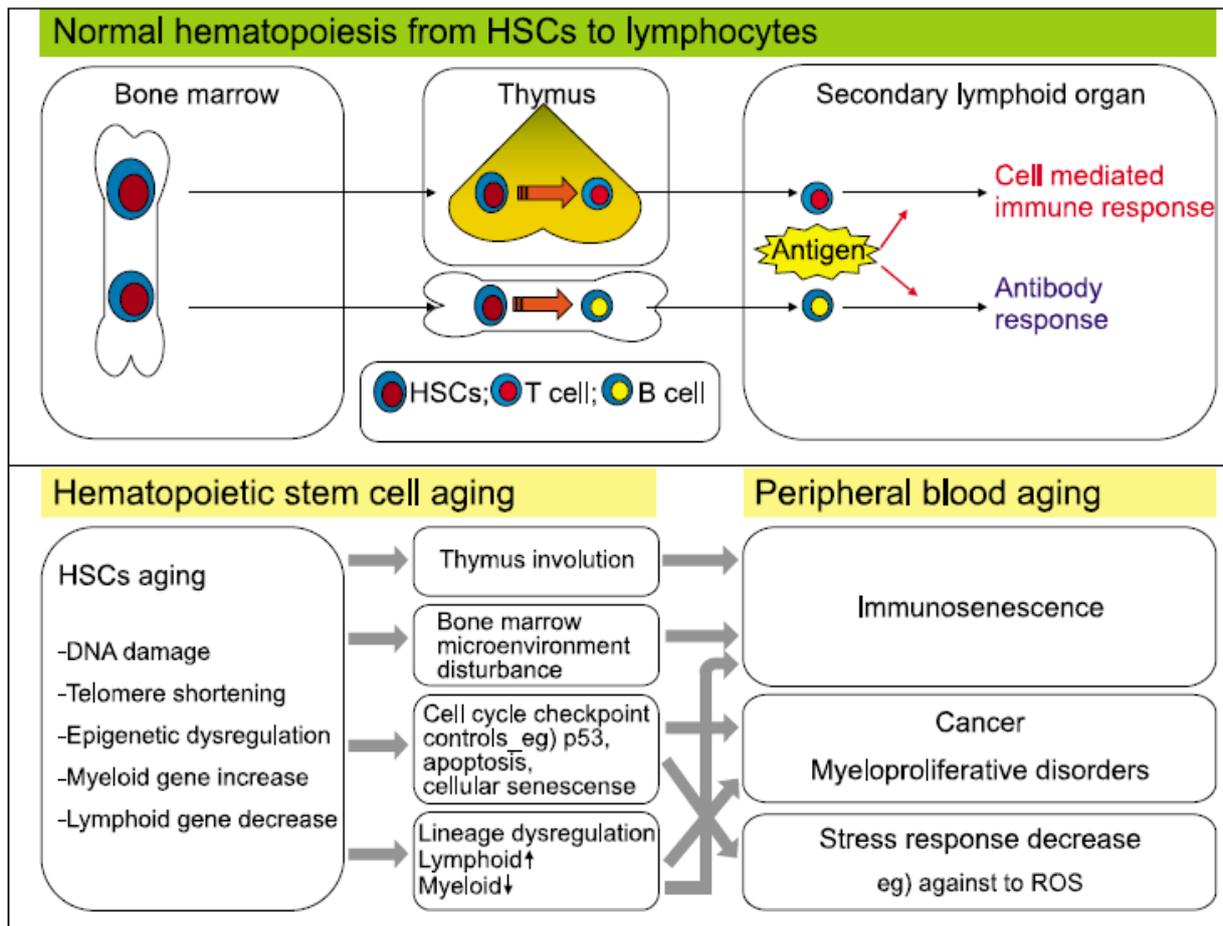


Figure 11. HSC aging consequences and effect on peripheral immunity. Figure adapted from (Kim et al., 2008).

1. Myeloid-Lymphoid phenotypic switch

HSC are the founder of the nascent immune system of the body by the generation of common myeloid and lymphoid progenitors. With aging, the production of myeloid cells increases while the production of lymphoid cell decreases. The change in lineage potential has been shown to be due to changes in gene expression at the stem cell level. For instance, LT-HSC aging is accompanied by systematic down-regulation of genes mediating lymphoid specification and function, and up-regulation of genes mediating myeloid specification and function (Rossi et al., 2005). Cho and his colleagues showed that aging is associated with a marked shift in the clonal composition of HSC in which lymphoid-biased HSC were lost, while myeloid-biased HSC were enriched in the aged mouse (Cho et al., 2008). However, myeloid-biased HSC from young and aged donors did not lose the capacity to generate lymphocytes. This phenotypic shift from the lymphoid to the myeloid lineage tends to skew the distribution of aged HSC and ultimately contributes to immune dysfunction and the decline of adaptive immunity in old age.

2. Leukemic manifestation

Aging promotes a leukemia pattern, increasing the risk of developing myelogenous disease, leukemia and tumors. Aged HSC express elevated levels of genes involved in leukemic transformation (Rossi et al., 2005). As such, Bcl3, a lymphoid leukemia gene was found to be up-regulated in old mice. Functional decline of aged HSC and alterations in cellular compartments that produce more myeloid cells may be involved in myeloproliferative disease and immune deficiency-related diseases in the elderly population (Warren and Rossi, 2009). Notably, while pediatric leukemia tends to be lymphoid in origin, leukemias that develop in old age often originate in myeloid compartments, suggesting that the malignant capacity of hematopoietic progenitors mirror changes in the lineage potential of HSC during aging (Kim et al., 2003; Rossi et al., 2005).

3. Molecular signature changes during aging

HSC aging is characterized by cellular intrinsic alterations. Using HSC purified from young and old mice, Chambers et al. analyzed over 14,000 genes, identifying 1,500 that were age-induced and 1,600 that were age-repressed (Chambers et al., 2007). Up-regulated genes were genes associated with the stress response such as apoptosis, inflammation, and protein aggregation were up-regulated, while those involved in the preservation of genomic integrity and chromatin remodeling were down-regulated. For example, MGST1, a gene that protects against oxidative stress, progressively increased with age; and BIK, an apoptotic gene, was positively correlated with age (Prall et al., 2007). I-Kb kinase/NF-Kb cascade, which is related to inflammation, increased with age (Chambers et al., 2007). FOXO protein, which is related to cell cycle arrest, increases with age to maintain HSC compartment (Greer and Brunet, 2005; Tothova and Gilliland, 2007). On the contrary, KU70, a DNA repair gene and part of the DNA-dependent protein kinase complex, is decreased with aging (Prall et al., 2007).

Several other studies investigated aging associated alterations in genes related to self-renewal and differentiation properties of HSC. For example, HOXB4, a gene that induces HSC self-renewal, increases HSC expansion capacity without functional impairment during aging (Antonchuk et al., 2002). Notch1, a gene that regulates HSC self-renewal, is reduced upon aging (Varnum-Finney et al., 2000). c-Myc, a gene that controls the balance between HSC self-renewal and differentiation and stimulates tumorigenesis, is increased with aging (Wilson et al., 2004; Wu et al., 2007a). TNF- α , a gene that is implicated in HSC self-renewal, expansion and hematopoiesis, is increased with aging (Zhang et al., 2007).

IV. DNA Damage Repair

A. DNA Damage

The integrity of DNA double helix is fundamental for the survival of cells, tissues, organs and the organism since it holds the whole genetic makeup. DNA is exposed to various insults throughout life that might be external or internal.

The external insults include environmental stressors. For example, ultraviolet (UV) radiation from the sun and various chemical reagents can react with DNA and induce nucleotide chemical modifications. Ionizing radiations (IR) generated by the cosmos, X-rays, and exposure to radioactive substances, as well as treatment with certain chemotherapeutic drugs, can induce base modifications, interstrand crosslinks, single- and double-strand breaks (DSBs), which can all lead to genomic instability (Mullaart et al., 1990).

The internal insults include genomic stressors. Due to the ongoing metabolic processes inside the cell, abundant endogenous lesions are continuously generated at an estimated frequency of approximately 20,000–50,000 lesions per cell per day in humans (Friedberg, 1995; Lindahl, 1993). For example, spontaneous hydrolysis reactions can occur resulting in depurination, depyrimidination and deamination of the DNA components. Oxidation of the DNA components can also occur leading to formation of ROS such as 8-oxoguanine, thymine glycol, cytosine hydrates and lipid peroxidation products. The endogenous lesions can also result in non-enzymatic methylation of the DNA components (Cadet et al., 2003; Friedberg et al., 2006).

The endogenous and exogenous lesions can result in persistent DNA damage that will perturb cellular processes and eventually lead to mutations and disturbed homeostasis, which may dramatically result in cancer and age-related diseases if left unfixed. Therefore living cells have developed several ways to contend with DNA damage including repair pathways and cellular responses.

B. DNA Damage Response

Several repair pathways are present in living cells and are in charge of repairing different types of DNA damage with varying fidelity and mutagenic consequences (Lombard et al.,

2005). The repair pathways include NER (nucleotide excision repair), BER (base excision repair), MMR (mismatch repair), HR (homologous recombination), and NHEJ (non-homologous end-joining). Damaged nucleotides are mainly repaired with high fidelity by the error-free excision repair pathways (NER, BER) and reversal of DNA damage pathway (MMR) resulting into the correct nucleotide sequence and DNA structure. Single- and double-strand breaks, caused by fracture of the sugar-phosphate backbone, are mainly repaired by the error-free HR pathway or by the error-prone NHEJ pathway (Friedberg et al., 2006) (Figure 12).

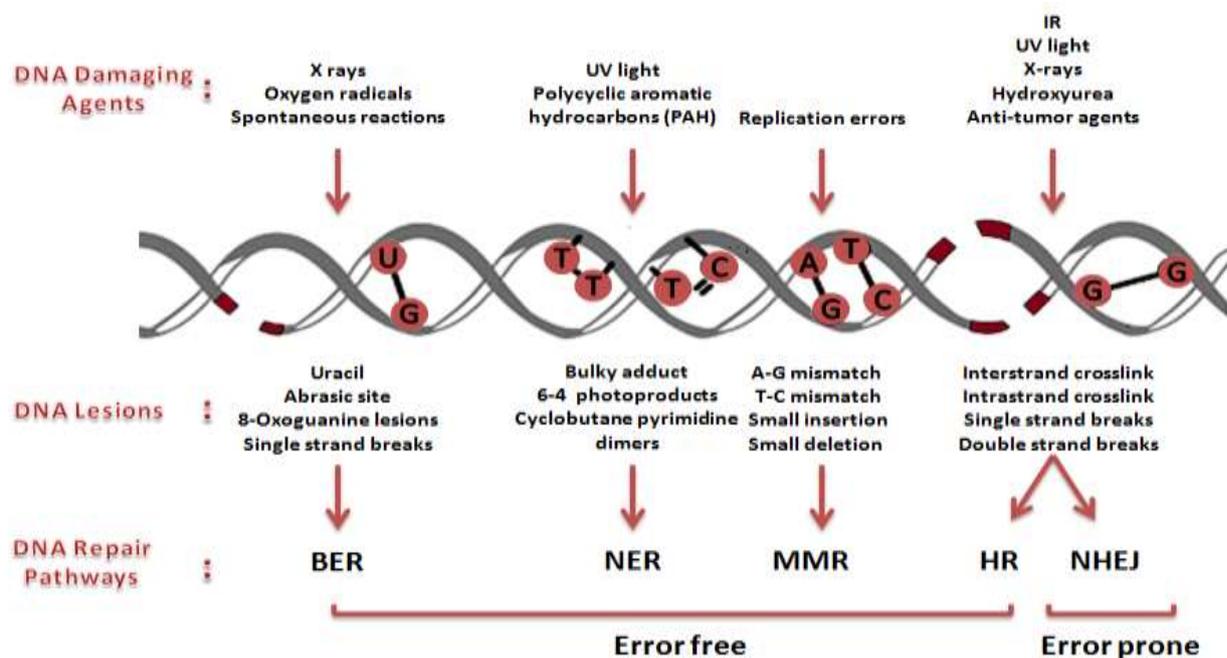


Figure 12. DNA repair pathways in mammalian cells. Different types of lesions caused by several DNA damaging agents are repaired by distinct repair mechanisms. Five DNA damage repair pathways exist in mammalian cells such that nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination (HR) are all error-free mechanisms while non-homologous end joining (NHEJ) is an error-prone mechanism. *Figure adapted from* (Blanpain et al., 2011; Melis et al., 2011).

To enhance the chances of cell survival, cells have evolved DNA damage tolerance mechanisms besides DNA repair pathways. DNA damage tolerance mechanisms include the error-prone translesion Synthesis (TLS) and the error-free template switching. These mechanisms enable cells to bypass lesions that normally block replication which rescue the cells even though it does not result in correct genomic maintenance (Gonzalez-Huici et al., 2014).

Moreover, irrespective of the type of DNA lesions and the repair mechanism, cells directly sense and respond to DNA damage by activating signaling pathways known as DNA-damage response (DDR). This evolutionary conserved cellular response is continuously present to insure the genomic stability of cells and include cell cycle checkpoint activation, scheduled cell death (apoptosis) and senescence (Bartek and Lukas, 2007).

At the molecular level, the first player in this response is p53 which becomes phosphorylated and stabilized and accumulates in the nucleus to upregulate its target genes. Therefore, depending upon the extent of DNA damage and the rapidity of its repair, the type of cell undergoing DNA damage and its cell cycle stage, the strength and the duration of *p53* activation and its transactivated genes, different outcomes are generated. First, cells can undergo transient cell-cycle arrest as a result of cyclin-dependant kinase inhibitor *p21* induction. The arrest of cell cycle progression in the different checkpoints will facilitate the efficiency of repair and damage tolerance by providing time for them to occur. Second, cells can undergo apoptosis as a result of induction of pro-apoptotic *bcl2* gene family members that include *bax*, *puma* and *noxa*. Apoptosis will program the cells with extensive DNA damage for cell death leading to attenuation of the risk of genomic instability and to maintenance of homeostasis. Third cells can undergo senescence through induction of the cyclin-dependant kinase inhibitor p16/Ink4a and the tumor suppressor gene *p19/ARF* and therefore preventing cells from proliferating and accumulating the DNA damage (d'Adda di Fagagna, 2008; Sancar et al., 2004) (**Figure 13**).

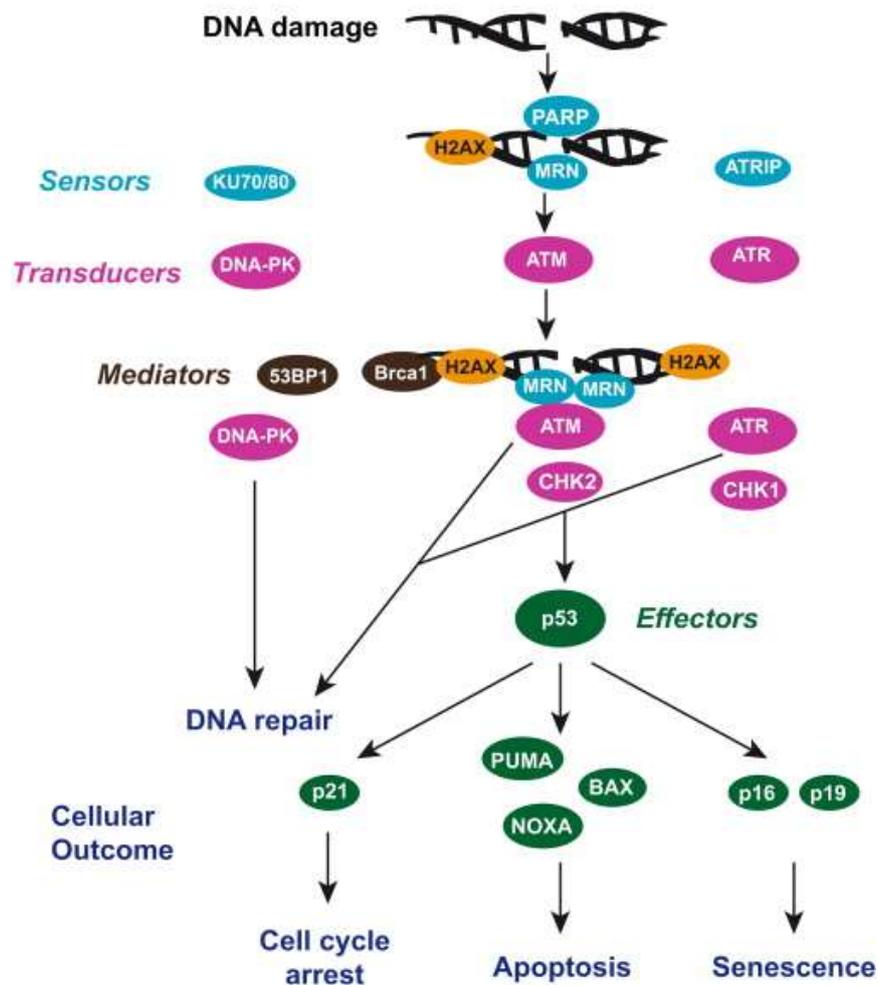


Figure 13. DNA-damage response pathways. Figure adapted from (Blanpain et al., 2011).

C. DNA Repair Mechanism

1. Double-Strand Break Repair

In eukaryotes, the repair of double-strand breaks (DSBs) is mediated by two either Homologous recombination (HR) or Non Homologous End-Joining (NHEJ). These two modes of DNA repair are not equally faithful and the decision of the damaged cell to use one of them is dependent on the presence of a homologous strand close to DSB and on the cell-cycle status. During the G_0/G_1 phase of the cell cycle, NHEJ pathway repairs DSBs through a process that is largely independent of terminal DNA sequence homology which produces junctions that can vary in their sequence composition. The absence of the intact template during repair can result in deletions, insertions, nucleotide modifications and chromosomal translocations. Thus NHEJ pathway repairs DSBs in error-prone manner. During the S- G_2/M

phase of the cell cycle, sister chromatids are very close, the HR pathway repairs DSBs through a process that is dependent of the other intact strand as a template. Thus HR pathway repairs DSBs in error-free manner (Li and Heyer, 2008; Lieber, 2010).

In addition to HR and NHEJ, there exists a third repair pathway of DSB considered to be an alternative form of NHEJ and is abbreviated is A-NHEJ. A-NHEJ function on simple end-joining principles but repair DSB slower than NHEJ. A-NHEJ is considered as a backup pathway since it gains functional relevance when the standard processes HR and NHEJ fail. In contrast to NHEJ which maintains the genomic stability, A-NHEJ is a major source of genomic instability. Indeed, A-NHEJ is more error prone than NHEJ since it can catalyze the joining of unrelated DNA molecules leading to more frequent sequence alterations at the junctions and a much higher probability of translocation formation (**Figure 14**).

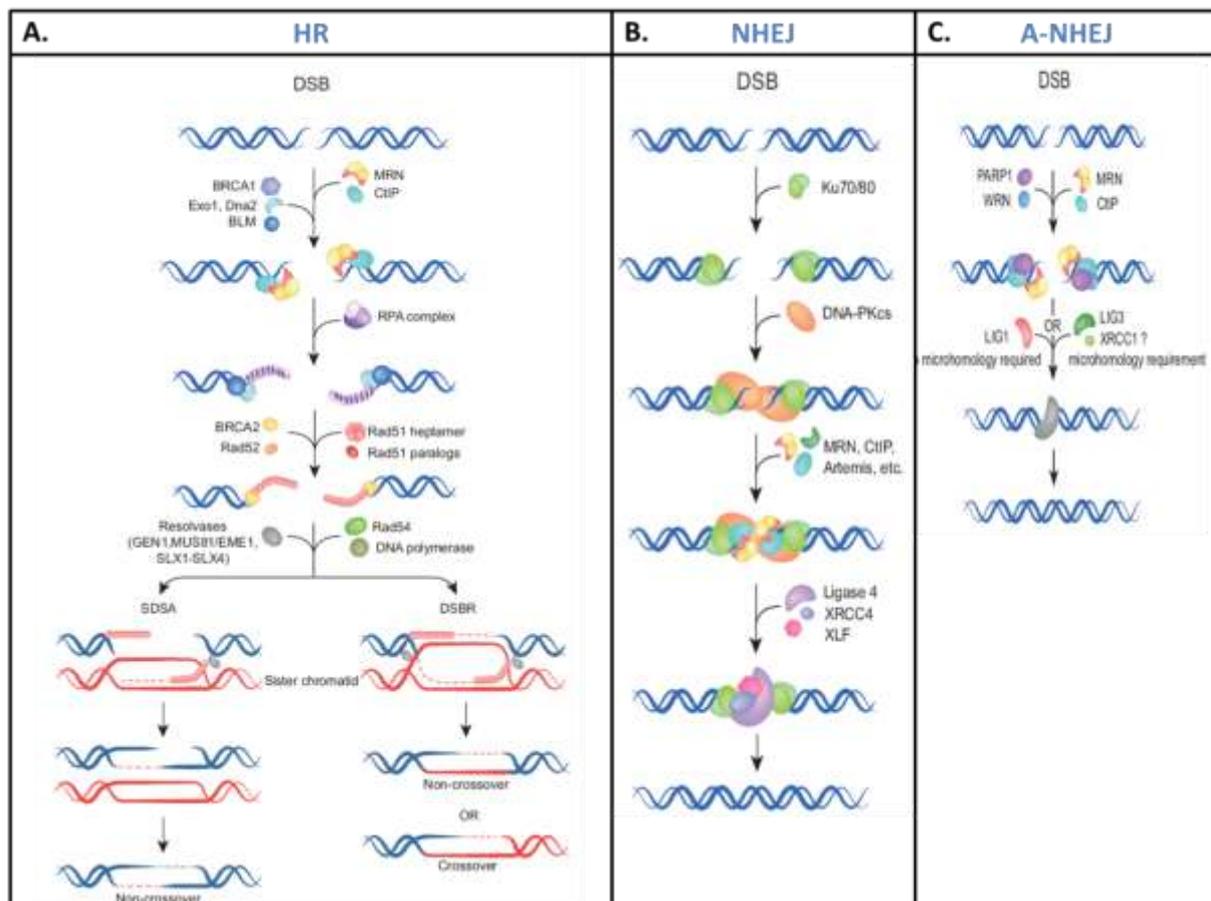


Figure 14. The mechanism of DSB repair. DSB can be repaired by homologous recombination repair (HR) (A), non homologous end-joining (B), and alternative non-homologous end-joining (A-NHEJ) (C). *Figure modified from (Dueva and Iliakis, 2013).*

a. Homologous repair pathway (HR)

The initial step in HR pathway is DNA nucleolytic end resection in which the DNA ends surrounding the DSB are trimmed by the heterotrimeric MRN complex (Mre11-Rad50-Nbs1) together with CtIP (RBBP8) and tumor suppressor protein BRCA1 creating short 3' single-stranded DNA (ssDNA) overhangs (Sartori et al., 2007). The resection is continued by the combined action of BLM helicase (Bloom syndrome, RecQ helicase-like) and Exo1 exonuclease and Dna2 helicase/nuclease (Nimonkar et al., 2008). Following end resection, single-stranded DNA tails are bound by Replication protein A (RPA) complex that confers its stability by removing disruptive secondary structures and protect it from nucleolytic cleavage. RPA is subsequently replaced by Rad51 in conjunction with several mediator proteins, such as Rad52, BRCA2, and a group of proteins known as Rad51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3) (Forget and Kowalczykowski, 2010). The Rad51-coated single-stranded DNA tail, also referred to as the Rad51 nucleoprotein filament, then executes the DNA sequence homology search, strand pairing and strand exchange. Rad54 supports the action of Rad51 by its branch migration activity. Next, DNA synthesis from the 3'-end of the invading strand is carried out by DNA polymerase η followed by successive ligation by DNA ligase I to yield a four-way junction intermediate structure known as a Holliday junction (McIlwraith et al., 2005). DSB repair by HR has been described in two different models. The synthesis-dependent strand annealing (SDSA) model describes the case where one 3'-ssDNA participates in the formation of a single Holliday junction but the double-strand break repair (DSBR) model describes the case where both 3'-overhangs participate in the formation of a double Holliday junction. Finally, the Holliday junctions are resolved by enzymes termed resolvases such as GEN1, MUS81/EME1, SLX1-SLX4 and the DNA sequence around the DSB is restored with or without cross-over resulting in the error-free correction of the DSB (Ip et al., 2008; Seki et al., 2006) (**Figure 14A**).

b. Non homologous end-joining pathway (NHEJ)

At the molecular level, NHEJ pathway is carried out by DNA-Protein Kinase (DNA-PK) which is a holoenzyme that consists of a catalytic subunit (DNA-PK_{CS}) and a DNA binding and regulatory subunit (Ku). The initiator step in this process involves the binding of Ku70/Ku80 heterodimer (Ku) to the exposed DNA termini of the DSB (Walker et al., 2001). Then, Ku-DNA complex recruits the quiescent protein kinase DNA-PK_{CS} leading to its activation by autophosphorylation which renders it capable of tethering the ends of the DSBs (DeFazio et al., 2002). However before proceeding to ligation of DNA, DNA ends may

require modification prior to ligation. For example, single-stranded overhangs present at DNA termini of DSB breaks are made ligatable through either resynthesis of missing nucleotides by DNA polymerases (Pol μ and Pol λ) (Lieber et al., 2008) or nucleolytic resection by NHEJ-specific nuclease Artemis characterized by endo- and exo-nuclease activities (Jeggo and O'Neill, 2002). After appropriate (or sometimes inappropriate) processing of the DNA termini, DNA ligase IV mediates the ligation of DNA ends in conjunction with its binding partner XRCC4. An additional factor, XLF (XRCC4-like factor), interacts with the XRCC4-DNA ligase IV complex to promote DNA ligation (Ahnesorg et al., 2006) (**Figure 14B**).

c. Alternative non homologous end-joining pathway (A-NHEJ)

A-NHEJ pathway has been reported to work in case of failure of mainly the NHEJ repair pathway which is abrogated prior to ligation by LIG4. Several of the early NHEJ factors may be present at the junction during the engagement of A-NHEJ. The proteins implicated in A-NHEJ are PARP1, the MRN complex and CtIP. It is suggested that end ligation occurs with one of the remaining ligases, LIG3 and LIG1. Noteworthy, it is thought that since LIG3 operates in other repair pathways together with XRCC1 and PARP1, its involvement in A-EJ implicates these factors in A-EJ as well (Dueva et al., 2013) (**Figure 14C**).

2. Mismatch Repair (MMR)

Before cell division and during DNA replication, the DNA polymerase is expected to make one mistake for each 10^7 incorporated nucleotides. The errors of replication, which lead to mutational insertions, deletions and base misincorporations, are repaired by MMR pathway. In addition, mismatches generated when single strands of non-identical duplexes are exchanged during homologous recombination are repaired by MMR pathway. In absence of efficient MMR, cells acquire mutator phenotype characterized by an elevated mutation frequency, microsatellite instability, and in some cases cancer predisposition.

The MMR pathway primarily involves three steps, recognition, excision and resynthesis. The recognition step is carried out by two major protein complexes called MutS which is responsible for mismatch recognition and MutL which is responsible of downstreaming MMR events by coupling to MutS complex (Modrich, 2006). Two MutS activities are present that function as heterodimers. MutS α represents the MSH2-MSH6 heterodimer and recognizes

base-base mismatches and small insertion deletion loops (IDLs) of one or two nucleotides. MutS β represents the MSH2-MSH3 heterodimer and recognizes larger IDLs. When MutS binds to DNA, it forms a sliding clamp able to translocate in either direction on the DNA until it recognizes a mismatch. This recognition alters the MutS conformation in an ATP-dependent manner, allowing recruitment of MutL in an ATP-dependent manner. Three MutL activities are present that also function as heterodimers. MutL α represents the MLH1-PMS2 heterodimer and possess the majority of MutL activity in humans and supports the repair initiated by both MutS α and MutS β . MutL β and MutL γ represent MLH1-PMS2 and MLH1-MLH3 heterodimers respectively and play minor roles in MMR. So when MutL α assembles with MutS, it acts as an endonuclease where it is able to recognize and excise the lagging strand from the mismatch. Exonuclease 1 (Exo1) is recruited to excise nucleotides in a 5'->3' direction while in the case of 3' excision, Exo1 recruits replication factor C (RFC) to perform a 3'->5' excision leaving a huge gap (Galio et al., 1999; Tran et al., 2004). The gap is resynthesized by DNA polymerase δ accompanied by at least two other proteins: proliferating cell nuclear antigen (PCNA) and replication protein A (RPA) (Kadyrov et al., 2007). RPA function to stabilize the single-stranded DNA and inhibit Exo1 to prevent further degradation. The PCNA factor is required during DNA synthesis for the processivity of the DNA polymerase and is also required during MMR for mismatch recognition by interacting with MutS and activation of the endonuclease. At the end, the remaining nick is sealed by DNA ligase I (**Figure 15**).

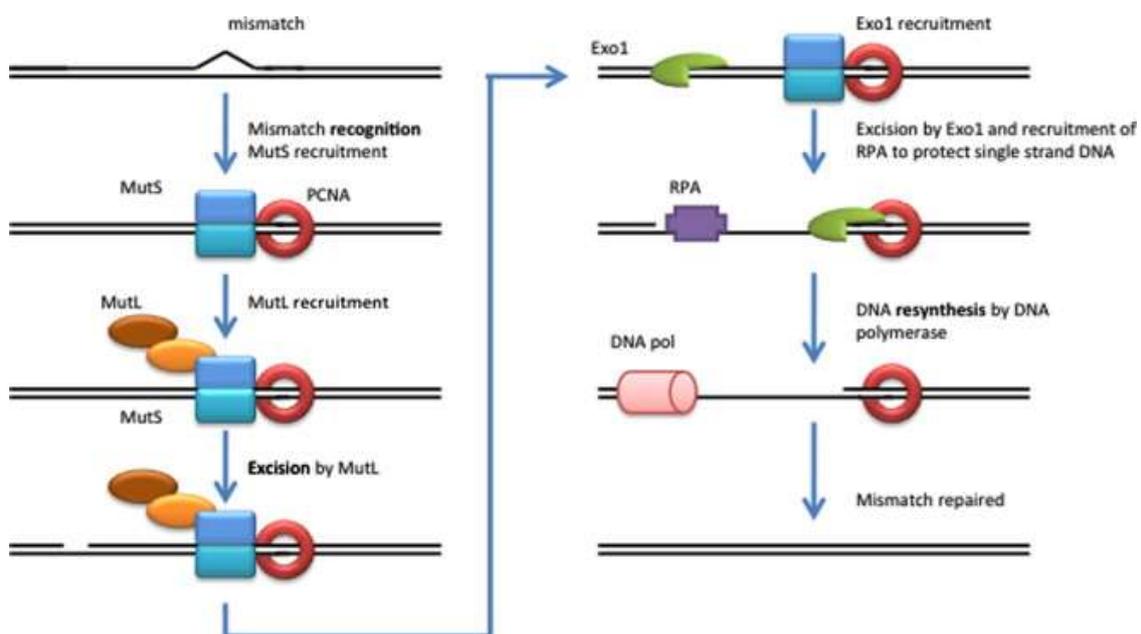


Figure 15. Schematic overview of MMR pathway. *Figure adapted from (Guillotin and Martin, 2014).*

3. Base Excision Repair (BER)

BER is an error-free repair mechanism that corrects base modifications, known to be non-bulky lesions, which are produced mainly by ROS-mediated DNA lesions generated by oxidation reactions and by alkylation or deamination lesions generated by spontaneous chemical reactions (Zharkov, 2008).

At the molecular level, BER is described as a highly coordinated pathway of consecutive enzymatic reactions. The initiator step of BER involves removal of the damaged base by lesion-specific DNA glycosylases which binds the altered deoxynucleoside in an extrahelical position by flipping the base and catalyzes cleavage of the base-sugar *N*-glycosidic bond (Hitomi et al., 2007). The release of the damaged base results in the formation of an apurinic/apyrimidic site (AP site). Then, AP sites are cleaved by AP endonucleases (APE1, 2) by hydrolyzing the phosphodiester backbone creating a single-strand break harboring 3' and 5' blocking regions (Abbotts and Madhusudan, 2010). To allow completion of the repair process, these blocked termini are restored by the aid of different DNA end-processing enzymes. After that, DNA strand breaks are repaired through DNA synthesis and ligation. This step is divided into short-patch subpathway where a single nucleotide is replaced and long patch BER subpathway where 2-10 new nucleotides are synthesized (Fortini and Dogliotti, 2007). The short-patch BER represents approximately 80–90 % of all BER and encompasses single nucleotide gap filling and successive ligation of the DNA ends by the complex of DNA ligase III and X-ray repair cross-complementing protein 1 (XRCC1) (Caldecott, 2003). Long-patch BER is normally only initiated as a result of 5' blocking lesions that are refractory to DNA polymerase β lyase activity. Long-patch BER demands several proteins associated with DNA replication, including DNA polymerase δ or ϵ , PCNA, RFC, FEN1 (flap endonuclease-1), and DNA ligase I. The flap structure is then removed by FEN1 and DNA ligase I sequentially seals the nick that has been relocated downstream of the original (**Figure 16**).

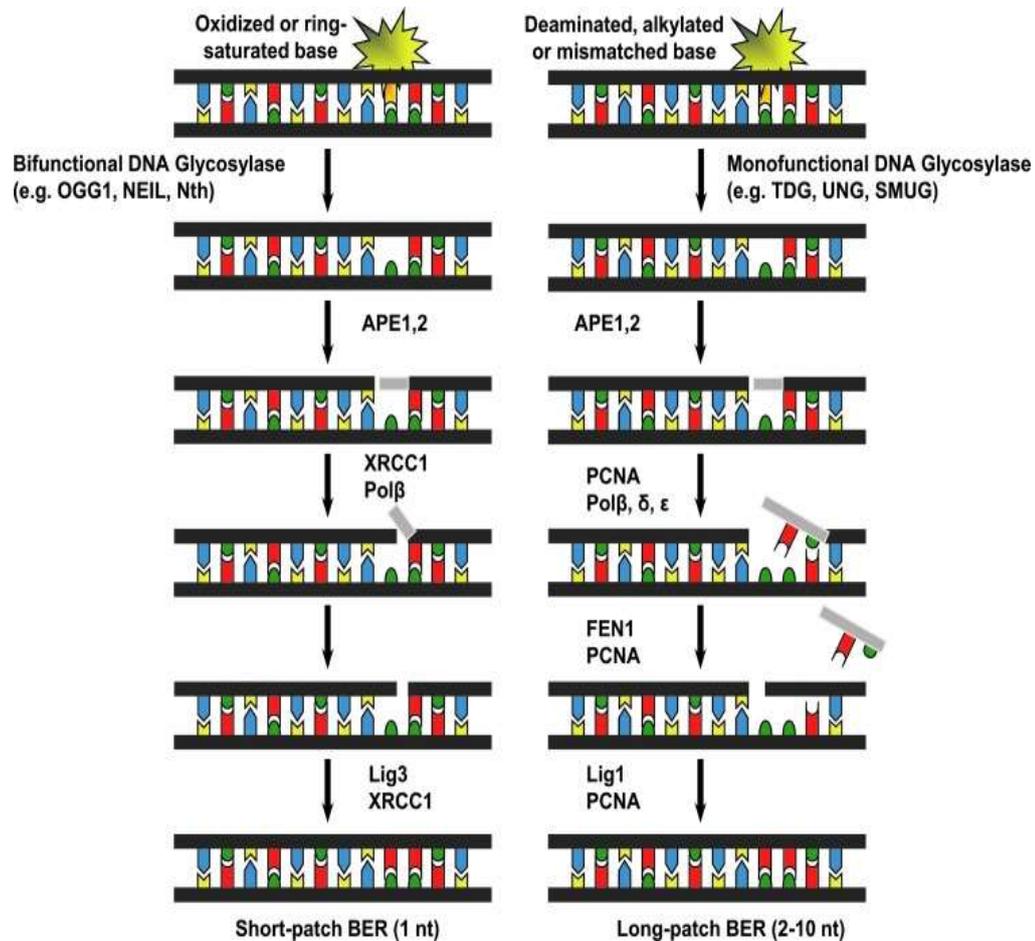


Figure 16. Schematic overview of the BER pathway. *Figure adapted from .*

4. NER

a. Definition

NER pathway is one of the most essential repair pathways due to the abundant targeting of bases and nucleotides in the genome. It is able to repair a wide range of DNA lesions and can restore the correct genomic information without arresting replication and transcription processes. It is an error-free mechanism that can repair a broad spectrum of primarily structurally unrelated bulky DNA lesions and helix-distorting damages induced by several damaging agents. For example, UV light is a physical DNA damaging agent that mainly produces cyclobutane pyrimidine dimers (CPDs) and pyrimidine-(6,4)-pyrimidone products (6-4PP) that are known to be the most relevant NER substrates (Lo et al., 2005). Numerous chemicals or alkylating agents can result into helix-distorting bulky adducts, for example polycyclic aromatic hydrocarbons (Boer and Hoeijmakers, 2000). NER is also considered as a back-up repair mechanism for removal of minor base damage induced by alkylating and

oxidizing agents that are usually repaired by the BER pathway. Indeed, the NER process requires the action of over 30 proteins in successive steps to eliminate the helix-distorting damage.

b. Modulation of NER in differentiation

As mentioned earlier, NER is a highly versatile repair system, capable of repairing a great variety of lesions including UV-induced pyrimidine dimers such as CPD and 6-4PP, bulky chemical adducts and intra-strand crosslinks. NER is already known to be composed of two major sub-pathways: transcription-coupled repair (TCR) and global genomic repair (GGR) (Hanawalt, 2002). Among the many repair pathways, NER appears to be especially modulated by differentiation in tissue-specific manner.

For instance, keratinocytes manifest an upregulated GGR relative to TCR upon differentiation and show an altered apoptotic response to UVB light that is independent of TCR (D'Errico et al., 2005). In contrast, some differentiated cells of blood and neural origin strongly downregulate GGR (Nospikel and Hanawalt, 2000a). Nospikel et al. have held work on NER susceptibility to differentiation in which they showed that terminally differentiated neurons displayed a strong impairment in GGR, while still proficiently repairing their active genes (Nospikel and Hanawalt, 2000b). However, studies of CPD repair during terminal differentiation of the human neuroteratoma cell line NT2 and of primary neurons (Nospikel and Hanawalt, 2000b, 2002) has led to discovery of another sub-pathway of NER which is termed as differentiation associated repair (DAR). DAR refers to the phenomenon that in certain cell types, when terminal differentiation is complete, there is a more rapid removal of certain lesions from non-transcribed strands of active genes in comparison with background GGR levels (**Table 1**).

Biological system	Damaging agent	Lesion assayed	NER in differentiated cell	References
Mouse/chick embryo	UV	UDS	Lower than fibroblasts	Sanes and Okun, 1972
Chick embryo retina	AAAF	Bulky adducts	Decreased	Karran et al., 1977
Rat neurons	UV	UDS	Low	Subrahmanyam and Rao, 1991
Mouse neuroblastoma	UV	Survival	Decreased	McCombe et al., 1976
Human neuroblastoma	BPDE	Bulky adducts	Decreased	Jensen and Linn, 1988
Mouse teratocarcinoma	UV	CPDs	Decreased	Czibula et al., 1997
Mouse embryocarcinoma	UV	CPDs	No repair	Rasko et al., 1993
Human NT2 neuroteratoma	UV	CPDs; (6-4) PPs	No repair; slower repair	Nouspikel and Hanawalt, 2000
Human NT2 neuroteratoma	BPDE	Bulky adducts	Slower repair	Nouspikel, 2007
Chick striated muscle	UV	UDS	Decreased	Stockdale, 1971
Rat L6 myoblasts	4NQO	Bulky adducts	Decreased	Chan and Walker, 1976
Rat L8 myoblasts	UV	CPDs	Initial Decrease	Ho and Hanawalt, 1991
Mouse 3T3-derived adipocytes	UV	UDS	Decreased	Tofilon and Meyer, 1988
Human lymphocytes	UV	UDS	Increased	Genter et al., 1984
Human HL60-derived granulocytes	UV	Survival, UDS	Increased	Nakamaki et al., 1989
Human HL60-derived macrophages	UV	CPDs	Decreased	Islas and Hanawalt, 1995
Human keratinocytes	UV	UDS	Decreased in upper layers	Eggset et al. 1983, Liu et al. 1983
Mouse keratinocytes	UV	CPDs, (6-4)PPs	Slower	Li et al., 1997

Table 1. Modulation of NER pathway in terminally differentiated cells. AAAF, N-acetoxy-acetylaminofluorene; BPDE, benzo[a]pyrene diolepoxide; CPDs, cyclobutane pyrimidine dimers; NER, nucleotide excision repair; UDS, unscheduled DNA synthesis (detects repair patches); 4NQO, 4-nitroquinoline-1-oxide; (6-4)PPs, (6-4) pyrimidine-pyrimidone photoproducts. *Table modified from (Nouspikel, 2007).*

c. NER pathway

NER is divided into two subpathways, GGR and TCR. GGR and TCR mechanistically initiate in a divergent manner, but after damage recognition both pathways proceed along the same processes. GGR recognizes and removes lesions throughout the entire genome rendering it a relatively slow process. However, 6-4PPs are rapidly cleared by GGR (Garinis et al., 2006).

On the other hand, TCR removes the lesions in the transcribed strand of active genes that blocks the transcription machinery. It is considered to be a fast and efficient process but is only initiated when transcription of a gene is blocked. Both pathways can generally be divided into four phases: DNA damage recognition, DNA unwinding, DNA damage incision, and DNA synthesis and ligation (**Figure 17**).

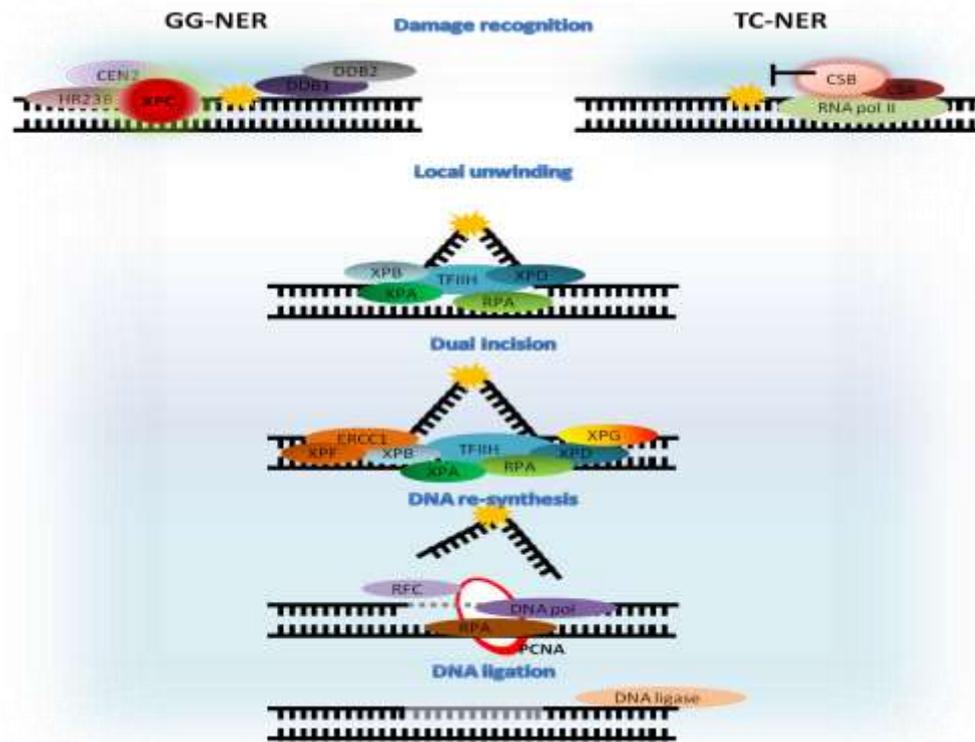


Figure 17. NER pathway. NER is divided into two subpathways that differ by damage recognition step. In GGR, XPC serves as damage sensor along with DDB2 while in TCR CSB and CSA recognize the stalled RNA polymerase in front of DNA damage. The two subpathways proceed along the same steps which include DNA unwinding by helicases, incision of DNA damaged strand by endonucleases, followed by re-synthesis of excised strand by DNA polymerases. Finally DNA ligase seals DNA ends thereby restoring the fully repaired DNA strand. *Figure designed based on information from (Melis et al., 2011, 2013a).*

In the DNA damage recognition phase, TCR is initiated when transcription is blocked by stalled RNA polymerase II, which is relieved through an assembled protein complex that includes two Cockayne Syndrome complementation group A (CSA) and B (CSB) proteins. CSB protein is a member of the SWI/SNF family of DNA-dependent ATPases and is implicated in chromatin remodeling but CSA is a cofactor for SCF-type ubiquitin ligase. CSB is responsible for the recruitment of the consecutive components of the NER machinery to the

damaged site while CSA is required to recruit the chromatin remodeling factors (Henning et al., 1995; Nakatsu et al., 2000; Tantin, 1998; Tantin et al., 1997).

On the other hand, in GGR, damage to non-transcribed regions of the genome is recognized by binding of XPC as an XPC–HR23B heterodimer (including CETN2) and XPE as a damage-specific DNA binding protein 2 (DDB2)–DDB1 heterodimer (Dip et al., 2004). The XPC-hHR23B complex is responsible for recruiting the preincision complex (Araújo et al., 2001). The binding affinity of XPC to the DNA correlates with the extent of helical distortion. For example, UV light induces primarily two kinds of lesions in the DNA: a majority of CPDs and a lesser amount (generally 1/3 to 1/4) of (6-4)PPs (Chandrasekhar and Van Houten, 2000). Both lesions are characterized by the formation of covalent bonds between adjacent pyrimidines on the same DNA strand, and differ only by the number and position of these bonds (Friedberg et al., 1995). Both lesions are repaired by NER, but with very different kinetics: (6-4)PPs are one of the best NER substrates, probably because they induce a strong DNA distortion of approximately 44° towards the major groove (Kim et al., 1995) and are thus rapidly repaired in most cell systems (Mitchell et al., 1985). CPDs, which only cause a 30° bend in DNA (Park et al., 2002), represent a much weaker substrate and are generally repaired much more slowly, in a process that requires the assistance of DDB at the global genome level (Tang et al., 2000). Therefore, XPC binds to 6-4PP adducts, that substantially distort the DNA structure, with higher specificity than for CPDs which is in turn recognized by DDB protein complex, thereby, complementing XPC action.

Therefore, XPC is indispensable for the recruitment of the subsequent NER complex to unwind the DNA. In fact, XPC protein contains several binding domains: a DNA binding domain, HR23B binding domain, centrin2 binding domain and a TFIIH binding domain. XPC itself has affinity for DNA and can initiate GGR. However, its stability requires assembly with centrin2 and HR23B proteins that inhibit polyubiquitinylation of XPC which inhibits its degradation by the 26S proteasome (Araki et al., 2001; Nishi et al., 2005). XPC is responsible for the recognition of various helix-distorting base lesions that do not share a common chemical structure. Biochemical studies have revealed that XPC recognizes a specific secondary DNA structure rather than the lesions themselves (Min and Pavletich, 2007; Sugasawa et al., 2001, 2002). XPC (together with DDB1 and DDB2) appears to scan the DNA for distortions by migrating over the DNA, repeatedly binding and dissociating from the double helix. When XPC encounters a lesion the protein changes its conformation and aromatic amino acid residues of XPC stack with unpaired nucleotides opposite the lesion,

thereby increasing its affinity and creating a conformation which makes it possible to interact with other NER factors (Hoogstraten et al., 2008).

In the second “DNA helix unwinding” phase, TCR and GGR subpathways overlap proceeding along the same process. The TFIIH complex which contains XPB and XPD helicases is recruited in order to open a bubble structure in the DNA helix, thereby, facilitating the entrance of the preincision complex to the lesion site (Giglia-Mari et al., 2004; Goosen, 2010).

In the third “DNA damage incision” phase, XPB and XPD helicases facilitate the partial unwinding of the DNA to allow the assembly of the pre-incision complex, formed by XPA, RPA and the structure specific endonucleases XPG and XPF–ERCC1 proteins, around the DNA damage. XPA is responsible for lesion verification and presents docking sites for XPG and XPF-ERCC1. While the single-strand DNA binding complex RPA facilitates the correct positioning of the repair proteins around the lesion serving as an organizational factor (Krasikova et al., 2010; Park and Choi, 2006). Both XPA and RPA are believed to protect the undamaged strand for incorrect incision (Hermanson-Miller and Turchi, 2002; Laat et al., 1998). After that, dual DNA incisions are made by XPG, at 3’ end, and ERCC1-XPF, at 5’ end (Staresincic et al., 2009), endonucleases result in the excision of a 24-32 nucleotide single strand fragment containing the damaged site (Hess et al., 1997).

In the fourth “DNA synthesis and ligation” phase, after the dual incision of damaged fragment, the gap is filled by DNA polymerases (Pol δ , Pol ϵ , and Pol κ) whose function is facilitated by proliferating cell nuclear antigen (PCNA), RPA, and replication factor C. In some cases, un-excised damage must be replicated by the action of Pol. The final step involves DNA ligation by DNA ligase that closes the 3’ nick leading to restoration of the original DNA fragment.

d. Outcomes of NER deficiency

In general, DNA repair is vital to all organisms and defect in one of the genes involved can result in severe syndromes or diseases by loss of genomic stability. Genomic instability can lead mainly to cancer by mutational activation of pre-oncogenes and inactivation of tumor suppressor genes and can also lead to other age-related diseases, such as neurological disorders like Huntington's disease and ataxias, by inactivation of indispensable genes in homeostasis maintenance (Friedberg et al., 1995).

NER is a complex pathway involving the direct interplay of around 40 genes in which a dozen of them have been found to be deregulated in NER-related human disease (Neumann et al., 2005). The remainder genes are thought to be either lethal if mutated or have mild clinical disease. However, many of the NER proteins are also components of dynamic multiprotein complexes, transcription factors, ubiquitinylation cofactors and signal transduction. Therefore, the deficiency of any of NER proteins might result in generation of complex clinical phenotypes due to unanticipated effect from other genes and proteins. Furthermore, genetic studies reveal a strong association between NER gene polymorphisms and the occurrence of many types of cancer. The involvement of NER genes in rare and severe syndromes underscores the vital importance of this pathway (Cleaver, 2005a) (**Figure 18**).

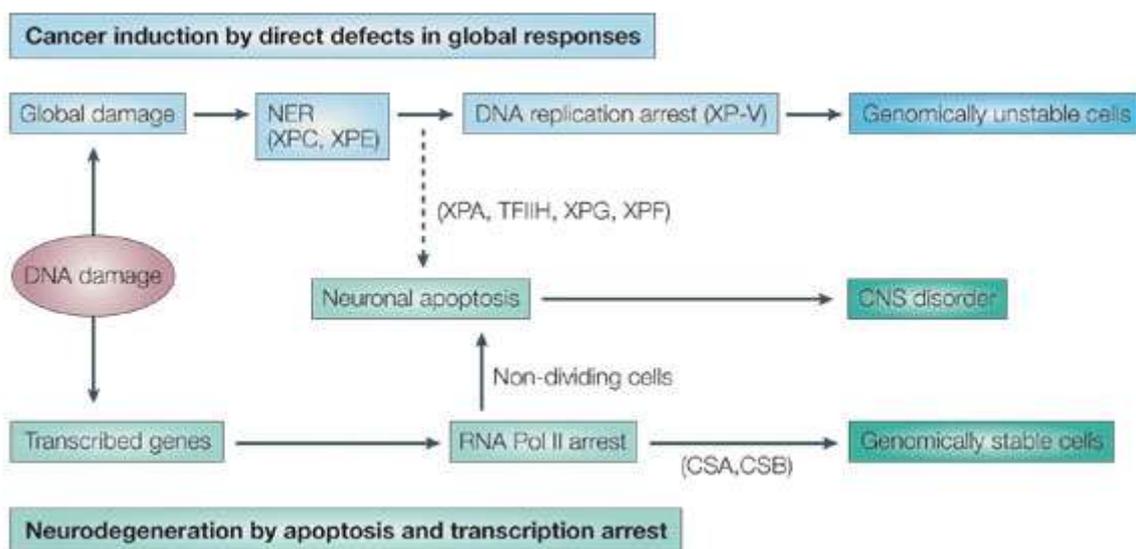
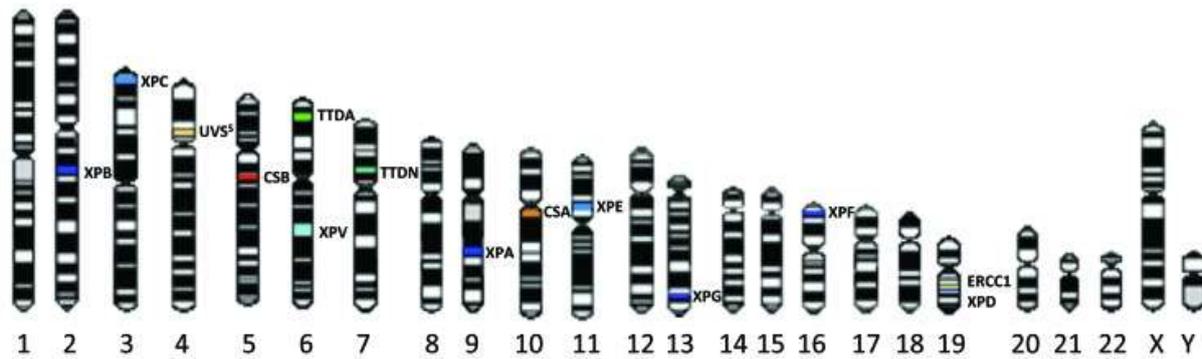


Figure 18. Outcomes of NER deficiency vary in link to the defective subpathway. Figure adapted from (Cleaver, 2005a).

Consequently, a variety of NER diseases are witnessed such as Xeroderma pigmentosum (XP), XP variant (XP-V), Cockayne syndrome (CS), Trichothiodystrophy (TTD), Cerebro oculofacial skeletal syndrome (COFS), mild ultraviolet (UV)-light-sensitive syndrome, and some diseases with combined symptoms of XP/CS and XP/TTD (Bootsma and Hoeijmakers, 1991). These diseases have complex overlapping symptoms associated with cancer, developmental delay, immunological defects, neurodegeneration, retinal degeneration and ageing. They represent a continuum with skin cancer alone at one extreme and neurodegeneration and developmental disorders at the other.

The most widely known of these diseases are XP, CS and TTD. Cancer is the hallmark of xeroderma pigmentosum (XP), and neurodegeneration and developmental disorders are the

hallmarks of Cockayne syndrome and trichothiodystrophy (Boer and Hoeijmakers, 2000; Cleaver et al., 2009; Friedberg et al., 2006; Mullaart et al., 1990). A distinguishing feature is that the DNA-repair or DNA-replication deficiencies of XP involve most of the genome, whereas the defects in CS are confined to actively transcribed genes. Since NER is the major defense against UV-induced DNA damage, all three syndromes are hallmarked by an extreme UV-sensitivity, of which XP ensues a highly elevated risk of developing skin cancer (Cleaver et al., 2009; Friedberg et al., 2006) (**Table 2**).



Repair mechanism	Gene	Chromosome	Protein size (aa)	No. of exons	Residual levels of repair (%)	Protein Function	Disease	Clinical features
Global genome repair	XPC	3p25.1	940	16	5-20	DNA damage recognition	XP	Skin cancer; No abnormal sunburn reaction; No neurological abnormalities
	XPE (DDB2)	11p11.2	427	10	>50	DNA damage recognition, E3 ligase	XP	
Transcription coupled repair	CSA (ERCC8)	5q12.1	396	12	100	RNA Pol II cofactor (Ubiquitinylation E3 ligase)	CS, UVSS	Mental retardation, microcephaly, and growth failure
	CSB (ERCC6)	10q11.21	1493	21	100	RNA Pol II cofactor (DNA-dependent ATPase)	CS, COFS, UVSS, DSC	
Common Pathway	XPA	9q22.33	273	6	2-5	Position of the DNA repair machinery (Damage verification)	XP, DSC	Exaggerated sunburn; Skin cancer; Mild to severe neurological abnormalities
	XPB (ERCC3)	2q14.3	782	15	3-7	Helicase 3'-5', TFIIH subunit	XP, XP/CS	Exaggerated sunburn; Skin cancer; Mild neurological abnormalities
	XPD (ERCC2)	19q13.3	760	23	25-50	Helicase 5'-3', TFIIH subunit	XP, XP/CS, TTD	Exaggerated sunburn; Skin cancer; No neurological abnormalities
	XPG (ERCC5)	13q33.1	1186	15	<2	Endonuclease 3' incision	XP, XP/CS	Exaggerated sunburn; Skin cancer; may present developmental abnormalities
	XPF (ERCC4)	16p13.12	916	11	18	Endonuclease 5' incision	XP, CS, COFS, XFE	Exaggerated sunburn; Skin cancer; No neurological abnormalities to severely affected patients
Post replication repair (TLS)	XPV (Pol η)	6p21.1	713	11	100	Bypass polymerase	XP	No abnormal sunburn reaction; Skin cancer; No neurological abnormalities

Table 2. Human chromosomal location and general description of the genes encoding proteins involved in NER and TLS including the consequences of their malfunction in terms of disease and clinical symptoms. Adapted from Cleaver, 2005b; Menck and Munford, 2014.

i. Cockayne syndrome (CS)

CS is a very rare genetic condition harboring a defective transcription-coupled repair. CS is divided into two types. CS type I is a classical form caused by defect in CSA gene located on chromosome 5 and presents during early childhood. CS type 2 is a more severe form caused by defect in DNA excision repair gene *ERCC6* located on chromosome 10 and presents at birth. Both types are rare autosomal recessive disorders that feature growth deficiency, premature aging, and pigmentary retinal degeneration along with a complement of other clinical findings. Cockayne syndrome is the only DNA repair disease that is not linked to cancer; but unfortunately, this disease is always fatal. Fatality usually occurs in early adolescence around 12 years of age (Laugel et al., 2010; Zhang et al., 2011a).

The major symptom of CS is growth failure, which includes progressive microcephaly in most patients in the first year. Neurologic examination shows increased or decreased muscle tone and reflexes; and some patients present with an unusual gait resulting from leg spasticity, ataxia, and contractures of the hips, knees, and ankles. Additional symptoms include pigmentary degeneration of the retina, sensorineural hearing loss, dental caries. However, the hallmark of this disease is that patients develop an aged appearance also called as progeria (Colella et al., 2000; Horibata et al., 2004; Nance and Berry, 1992).

ii. Trichothiodystrophy (TTD)

Trichothiodystrophy (TTD), a rare group of autosomal recessive disorders of DNA repair with a wide range of phenotypic expression, is unified by the presence of sulfur-deficient brittle hair (Faghri et al., 2008). Most cases of this disease result from mutations in one of three genes: *ERCC2*, *ERCC3*, or *GTF2H5* whose encoded proteins are subunits of TFIID complex. As previously mentioned, TFIID complex is involved in the repair of DNA damage but also plays an important role in gene transcription. Therefore, mutations in the *ERCC2*, *ERCC3*, or *GTF2H5* genes reduce the amount of TFIID complex within cells, which impairs both DNA repair and gene transcription leading to generation of variety of symptoms (Botta et al., 2009).

The signs and symptoms of trichothiodystrophy vary widely. Mild cases may involve only the hair. More severe cases also cause delayed development, significant intellectual disability, and recurrent infections. Other features of trichothiodystrophy can include photosensitivity leading to dry scaly skin (ichthyosis), congenital cataracts and skeletal abnormalities. Severely

affected individuals may survive only into infancy or early childhood (Itin et al., 2001; Porto et al., 2000).

iii. Xeroderma pigmentosum (XP)

Xeroderma pigmentosum (XP) was the first human NER-deficient disease to be discovered and refers to parchment pigmented skin (Cleaver et al., 2009). The incidence frequency of XP is approximately 1:250,000 in Western Europe and USA and 1:40,000 in Japan where the rate is much higher. XP is a rare disorder transmitted in an autosomal recessive manner and is characterized by photosensitivity, pigmentary changes, premature skin aging, and malignant tumor development. These manifestations are due to a cellular hypersensitivity to UV radiation resulting from a defect in DNA repair (English and Swerdlow, 1987).

XP is caused by a defective NER leading to deficient repair of damaged DNA mostly by UV radiation. XP disease is an archetype of expanding family of human NER-diseases. Before the NER genes were cloned and identified, patients were assigned to complementation groups by cell-fusion experiments. Once the mutated genes were identified and sequenced, they were named after the complementation group with which they associate. Indeed, there are seven complementation groups that have been identified to be involved in XP disease. These include *XP-A* through *XP-G* that are caused by mutations in genes that code for *XPA* to *XPG* proteins. One variant form called *XPV*, caused by mutation in a gene that codes for polymerase- η was also characterized. *XP-A* and *XP-C* are the most common complementation groups of XP. These genes play key roles in GGR and TCR. The heterogeneity in symptoms is correlated to the genetic heterogeneity in XP patients. For example, *XP-A*, *XP-B*, *XP-D* and *XP-G* patients are in general severely affected, showing neurological disorders, possibly because these patients are defective in both the GGR and TCR subpathways. On the other hand, *XP-C* and *XP-E* patients rarely develop neurological disorders and show higher survival rates after UV exposure than *XP-A* and *XP-D* cells possibly because these patients are only defective in GGR (Cleaver, 2005a; Friedberg et al., 1995, 2006).

XP patients show three major types of abnormalities. First, cutaneous abnormalities represent the hallmark of this disease and appear in the early childhood at the age of 1.5 years. It includes diverse symptoms such as photosensitivity, cutaneous atrophy and telangiectasia, actinic keratosis and malignant skin neoplasms that are the most prevalent appearing at the age of 8 years. Interestingly, XP is associated with a more than 1000-fold increase in risk of

developing skin cancer, comprising basal and squamous cell carcinomas and to a lesser extent melanomas (Friedberg et al., 2006). Second, ocular abnormalities appear at the age of 4 years and include conjunctival and corneal problems, impaired vision, photophobia and ocular neoplasms. Third, neurological abnormalities progressively appear early after 6 months of birth and include slow growth, abnormal motor activity, areflexia, impaired hearing, abnormal speech, microcephaly and low intelligence that is the most prevalent.

Physically, XP typically passes through 3 stages noting that the skin is healthy at birth. The first stage appears after age 6 months. Diffuse erythema, scaling, and freckle like areas of increased pigmentation characterize this stage. With progression of the disease, the skin changes appear on the lower legs, the neck, and even the trunk in extreme cases. While these features tend to diminish during the winter months with decreased sun exposure, as time passes, these findings become permanent. The second stage is characterized by poikiloderma. Poikiloderma consists of skin atrophy, telangiectasias, and mottled hyper pigmentation and hypopigmentation. The third stage is heralded by the appearance of numerous malignancies, including squamous cell carcinomas, malignant melanoma, basal cell carcinoma, and fibrosarcoma. These malignancies may occur as early as age 4-5 years and are more prevalent in sun-exposed areas.

In addition, several immunologic abnormalities have been described in the skin of XP patients caused by UV-B radiation that imposes immunosuppressive effects that may be involved in the pathogenesis of XP. Clinical studies of the skin of patients with XP indicate prominent depletion of Langerhans cells induced by UV radiation. Various other defects in cell-mediated immunity have been reported including impaired cutaneous responses to recall antigens, decreased ratio of circulating T-helper cells to suppressor cells, impaired lymphocyte proliferative responses to mitogen, impaired production of interferon in lymphocytes, and reduced natural killer cell activity (Kraemer, 1997).

Besides that XP patients have a 10–20 fold increased risk of developing internal cancers (Kraemer et al., 1984). However, the severe and early onset of skin cancer in humans suffering from XP may overshadow potential tumors in other tissues such as the liver and lung, which were found in NER-deficient mice. It is believed that oxidative DNA damage is a contributing or possibly even a driving factor to internal cancer development in XP patients since internal tissues are generally not exposed to UV, and are probably scarcely exposed to chemicals that induce bulky adducts. For example, colorectal, bladder, and lung carcinogenesis have been correlated with increased oxidative stress levels. Another example is that the analysis of the internal tumors of XP-C patients indicated that unrepaired oxidative

DNA lesions most likely cause mutations. In addition, internal tissues can be subjected to harmful environmental agents or their reactive metabolites that can cause DNA damage (Table 3).

XP features	Median Age of onset	Symptoms	Frequency (%)
Cutaneous abnormalities	1.5 years	Photosensitivity	19
		Cutaneous atrophy	23
		Cutaneous telangiectasia	17
		Actinic keratosis	19
		Malignant skin neoplasms	45
Ocular abnormalities	4 years	Conjunctival injection	18
		Corneal abnormalities	17
		Impaired vision	12
		Photophobia	2
		Ocular neoplasms	11
Neurological abnormalities	6 months	Low intelligence	80
		Abnormal motor activity	30
		Areflexia	20
		Impaired hearing	18
		Abnormal speech	13
		Abnormal EEG	11
		Microcephaly	24
Developmental defects	Associated with neurological defects	Slow growth	23
		Delayed secondary sexual development	12

Table 3. Most abundant XP features, symptoms, average onset and frequency. *Adapted from* (Friedberg et al., 2006; Melis et al., 2011).

V. Xeroderma Pigmentosum group C (XPC)

A. XPC discovery

XPC was first identified as one of the seven complementation groups of xeroderma pigmentosum (XP) in 1933 and characterized in 1991 (Bootsma and Hoeijmakers, 1991). It is the most common form of XP in the white population, accounting for over a third of all cases in this group (Li et al., 1993). This complementation group corresponds to mutations in the gene coding for the XPC protein that is localized on the short arm of chromosome 3 on band 25.1. XPC protein, constituting of 940 amino acids, is majorly known as an essential DNA damage recognition protein of the GGR pathway where it harbors domains that can bind to damaged DNA and repair factors.

XPC patients have been shown to exhibit diminished DNA repair capacity, abnormal skin lesions, freckling, atrophy, telangiectasia, hypopigmentation, actinic keratosis, multiple skin cancers (Khan et al., 2004). Those symptoms can be explained by the role of XPC as a sensor of DNA damage in the GGR pathway. However, some XPC patients exhibited neurological symptoms such as systemic lupus erythematosus (Hananian and Cleaver, 1980), hyperactivity, and autistic features (Khan et al., 1998) and also exhibited ophthalmological symptoms such as the clouding of the cornea, prominent vascular growth on the conjunctiva, and loss of lashes. These studies strongly suggested that XPC is implicated in various roles in the cell other than its role in DNA damage recognition in GGR.

B. XPC binding characteristics

Upon occurrence of DNA damage such as CPD or 6-4PPs, XPC polypeptide (125 kDa) binds to DNA in association with Rad23B (58 kDa) and centrin2 (18 kDa) inducing a kink of 39–49° in the nucleic acid backbone and the resulting sharp bend is accompanied by partial melting of the duplex extending over 4–7 base pairs. Rad4 protein is a yeast ortholog of human XPC sharing ~40% similarity and ~25% identity in their sequence. Crystallization of parts of Rad4 protein has allowed the examination at atomic resolution of the interaction between XPC and DNA duplex. Apparently, Rad4 protein binds to the substrate in bimodal manner. One portion of Rad4 protein, consisting of its large transglutaminase homology domain (TGD) in conjunction with a short β -hairpin domain (BHD1), forms a C-clamp-like structure that interacts with 11 base pairs of native double-stranded DNA located on the 3'

side of the lesion. In the N-terminal region, Rad23 polypeptide interacts with several residues mapping to the beginning and the end of the TGD region. Another portion of Rad4 protein, composed of the β -hairpin domains BHD2 and BHD3, folds into a hand-like structure that associates with a 4-nucleotide DNA segment at the lesion site. The BHD2/BHD3 motif provides aromatic side chains that envelop the flipped-out normal residues. Interestingly, this central DNA-binding region of XPC protein shares $\sim 75\%$ amino acid similarity and $\sim 30\%$ identity with the oligonucleotide/oligosaccharide-binding fold (OB-fold) of RPA-B, one of the single-stranded DNA-binding motifs of human RPA. In human XPC, the N-terminal region is responsible for an association with XPA in which the TGD segment of human XPC protein is separated into two individual parts by a disordered ~ 180 residue insertion. However, the carboxy-terminal tail of XPC protein harbors domains that interact with centrin-2 (residues 847–863) and TFIIH (residues 816–940) (Clement et al., 2010) (**Figure 19**).

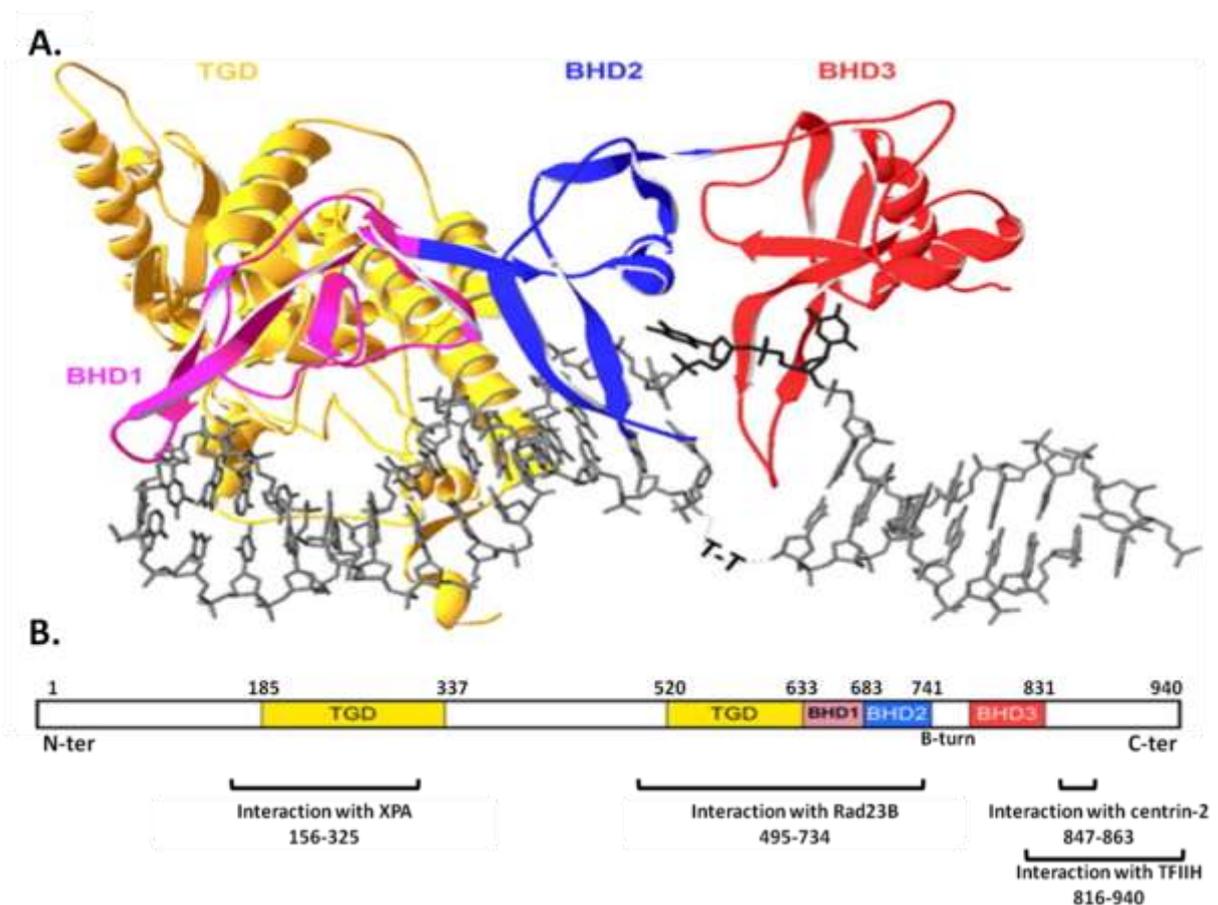


Figure 19. Structure of XPC protein inferred from the yeast Rad4 homolog. A. Ribbon diagram of the crystal structure of a Rad4 protein fragment (residues 123–632) in complex with heteroduplex DNA carrying a CPD lesion. Multiple Rad4 domains interact with the DNA substrate: TGD (gold), BHD1 (magenta), BHD2 (blue) and BHD3 (red). T-T denotes the CPD, which is totally expelled from the duplex. B. Scheme of the homologous domains in the human XPC sequence and the domains involved in interactions with Rad23B, XPA, centrin-2 and TFIIH. *Figure adapted from (Clement et al., 2010).*

C. XPC post-translational modifications

XPC-complex interactions with cellular components and novel functionalities have been difficult to pinpoint solely by transcriptional analysis which lead scientists to study post-translational modifications of XPC. XPC is a target for several post-translational modifications during the course of its role in GGR which plays an important role in précising its function. Ubiquitinylation modification of XPC is the best-known. It is carried out by the DDB2-associated DDB1-CUL4-ROC1 complex which increases the affinity of XPC to damaged DNA and is potentially involved in the handoff of 6-4PP repair from DDB2 to XPC (Sugasawa, 2006a). XPC is also subjected to sumoylation, which has been proposed to protect XPC from degradation after UV irradiation. Recently, it has been indicated that ubiquitinylation of XPC after sumoylation by RNF111 serves to promote NER (Poulsen et al., 2013). Furthermore, phosphorylation modification of XPC has been found by ataxia telangiectasia mutated (ATM) and Rad3-related (ATR) kinase in response to UV damage. The phosphorylation of XPC activates it along with p53 and XPA to arrest cell cycle progression, stimulate DNA repair, or initiate apoptosis. However, following the completion of DNA repair, there must be active mechanisms that restore the cell to a pre-stressed homeostatic state. Thus it has been reported that the serine/threonine wild-type p53-induced phosphatase 1 (WIP1) dephosphorylate XPC after the repair of the UV-induced DNA damage which will lead to its inactivation and to suppression of NER (Nguyen et al., 2010).

D. Functions of XPC

XPC has been the spotlight of abundant studies which aimed to unravel its function in both humans, by the study of XPC patients and human cell lines, and mouse by the study of *Xpc* knockout (KO) mouse models. In fact, there exist two *Xpc* KO mouse models reported by Sands et al. year 1995 and by Cheo et al. year 1997. Both models were generated by homologous recombination-mediated gene replacement using embryonic stem cell technology. In the *Xpc* KO mouse model of Sands et al., 4.5 kb of the mouse *Xpc* gene was deleted which involves region spanning exons 3-6 and which eliminates a coding portion of the mouse gene corresponding to bases +59 to +546 of the published human XPC cDNA. The mutant allele in these animals expressed a truncated mRNA containing a deletion, frameshift and stop codons. The chimeric animals were C57BL/129 hybrids. The generated *Xpc* KO mice are viable and do not exhibit an increased susceptibility to spontaneous tumor generation at one year of age. However, cells from these animals manifested increased sensitivity to UV

light and also demonstrated a predisposition to skin cancer induced by exposure to UV light (Sands et al., 1995). In the *Xpc* KO model of Cheo et al., 1.2 kb of the mouse genomic *Xpc* gene was deleted containing exon 10 and a portion of each of the flanking introns with a POLII-NEO selectable marker. The mutant alleles expressed truncated transcripts which are expected to encode a polypeptide shortened by 300 C-terminal amino acids with the addition of 27 missense amino acids noting that C-terminal region of the XPC polypeptide is indispensable for functional activity and is required for interaction with both the HHR23A and HHR23B gene products. The chimeric animals were C57Bl/6 hybrids. The generated *Xpc* KO mice do not show obvious abnormalities under standard laboratory conditions, however, under exposure to UV light, cells from those mice express increased sensitivity, reduced repair synthesis of DNA indicating an NER defect (Cheo et al., 1997).

The intense research on XPC in the past years has lead to discovery of the different functions of XPC mainly in NER as sensor of DNA damage, in redox homeostasis and in cell cycle checkpoint which will be discussed in details in the following sections (**Figure 20**). Interestingly, a novel study by Lubin et al. have detected the interactome of XPC using a high-throughput Yeast Two Hybrid screening (Lubin et al., 2014). Out of the discovered protein interactome, 12% had roles in DNA repair and replication, 14% had roles in proteolysis and post-translational modifications, 8% had roles in transcription regulation, 20% had roles in signal transduction and the majority 32% had roles in metabolism.

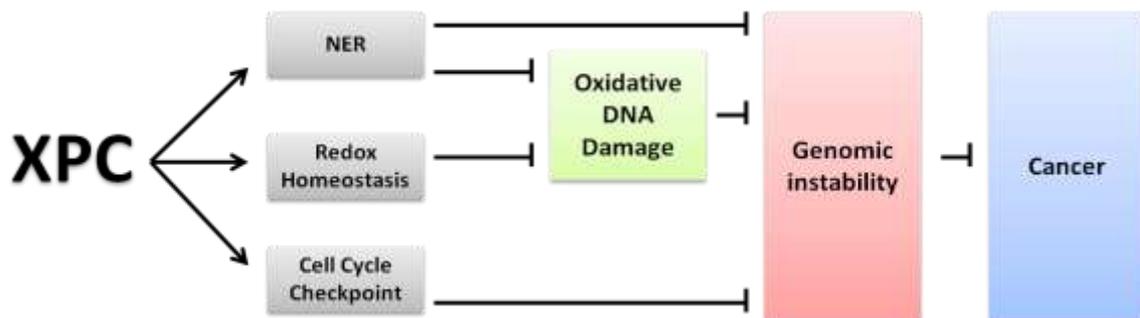


Figure 20. XPC functions and their subsequent roles in inhibition of oxidative DNA damage, genomic instability and cancer. *Figure designed based on information from (Melis et al., 2011).*

1. Bulky DNA Damage Repair

The XPC complex is the main early damage detector in GGR pathway and serves to stabilize the low-affinity XPA and RPA proteins that subsequently bind at the damaged site. It recognizes a large spectrum of DNA lesions characterized by DNA distortions that do not share a common chemical structure. Many biochemical studies revealed that XPC recognizes a specific secondary DNA structure rather than the lesions themselves (Min and Pavletich, 2007; Sugasawa et al., 2001, 2002). By changing the spatial conformation, XPC increases its affinity to interact with other NER factors. Min and Pavletich suggested that XPC binds opposite to the lesion site and flips the damaged bases out of the helix structure, providing an explanation for the broad specificity of GGR damage recognition (Min and Pavletich, 2007). Highly distorting lesions provide sufficient opening of the DNA strands so, XPC can be dispensed with in cell-free systems of NER (Sancar, 1996; Wood, 1997). XPC itself has affinity for DNA and is able to initiate GGR *in vitro*, but its functionality is enhanced when hHR23b and centrin2 are added (Araki et al., 2001; Nishi et al., 2005). XPC (together with DDB1 and DDB2) appears to scan the DNA for distortions by migrating over the DNA, repeatedly binding and dissociating from the double helix (Hoogstraten et al., 2008). Thus the XPC protein has several binding domains: a DNA binding domain, an HR23B domain, centrin2 binding domain and a TFIIH binding domain. In the absence of DNA damage, XPC protein is omnipresent in the nucleus and consistently associates and dissociates from DNA. Therefore, in the case of absence of XPC, the residual NER is biased towards transcribed regions, but the total amount of residual repair appears to involve large stretches of DNA, greater than the amount represented by the active genes alone (Boon et al., 2004; Consortium, 2004). Therefore, in these XPC patients, the cells can accumulate mutations in non-transcribed parts of the genome, leading to neoplastic transformation.

2. Carcinogenesis

The strong implication of XPC in carcinogenesis process has been the focus of research from long time ago. XPC defects have been found in many types of cancer as revealed by studies on *Xpc* KO transgenic mice and by other genetic studies (Miccoli et al., 2007).

a. Skin tumors

The strongest milestone correlating XPC to skin cancer development was the fact that XPC defect in humans gave rise to XP disease (Neumann et al., 2005; Wood et al., 2001) which shows hypersensitivity to UV light, skin atrophy, actinic keratoses and increased risk of skin cancer by 1000-fold (Kraemer et al., 1987). Other studies in mouse correlated XPC defect with skin carcinogenesis. *Xpc* KO mouse model, referred to as *XPC*, which is only defective for GGR and not for TCR, showed highly predisposed to UV radiation-induced skin cancer same as in humans (Berg et al., 1998; Cheo et al., 1996, 2000; Friedberg et al., 1999; Sands et al., 1995). Even heterozygous *Xpc* mice showed higher susceptibility to UV-induced skin cancer in comparison to their wild type littermates which was not the case in *Xpa*^{+/-} mice (Friedberg et al., 2000).

b. Internal tumors

Several studies have showed that XPC defect is implicated in development of internal tumors. However, the incidence cases of internal tumors due to XPC defect remained limited and less prevalent than occurrence of skin tumors due to the fact that internal tissues are not exposed to UV and are scarcely exposed to chemicals that induce bulky adducts. The first clue of XPC implication in internal tumors was that, in XP patients, an increased risk of developing internal cancer by 10- to 20-fold has been reported (Kraemer et al., 1987). A later study, XPC defect was correlated with lung, liver, bladder, and colorectal cancer in mice. Two separate survival studies were carried for the *Xpc* KO model. The first study by Hollander and his colleagues was performed in a mixed genetic (75% C57BL/6J, 25% 129) background where the wild type mice were not genetically related to the *Xpc* KO mice (Cheo et al., 1999; Hollander et al., 2005a). The study did not reveal any decrease in survival for *Xpc* KO mice compared to wild type mice, even though *Xpc* KO mice showed an extremely high and significantly increased lung tumor incidence. Both at the time of death and at the intercurrent age of 16–17 months 100% of the *Xpc* KO mice harbored lung tumors.

The second study was performed in a congenic (C57BL/6J) background, which also showed a significant increase in lung and liver tumors (Melis et al., 2008a). In contrast, *Xpa*-deficient mice spontaneously had a significantly increased tumor incidence in the liver, but not in the lung (Melis et al., 2008a). Here, *Xpc* KO mice show a divergent tumor spectrum from the *Xpa*-deficient mice in the same genetic C57BL/6J background. The additional increase in lung tumor development in two independent spontaneous survival studies indicates that XPC is involved in other pathways besides NER. Moreover, three recent articles showed an association of XPC polymorphisms with increased lung cancer risk (Hu et al., 2005; Lee et al., 2005). In addition, XPC protein plays an important role in preventing the occurrence of

bladder cancer (Chen et al., 2007). Recent studies demonstrate reduced levels of XPC protein in tumors for a majority of bladder cancer patients.

3. Cell metabolism

Several studies have assigned XPC to a more elaborate role in DNA repair besides the removal of chemically or UV induced bulky adducts that trigger NER. A secondary role in cell metabolism has been discovered for XPC by its implication in ROS generation, oxidative DNA damage accumulation, and redox homeostasis.

a. *In vitro* studies

Concerning *in vitro* experiments, a study performed on XPC-deficient cells compared to control fibroblasts showed higher ROS levels (Fréchet et al., 2008a). Another study on primary keratinocytes and fibroblasts derived from XP-C patients showed a hypersensitive to DNA-oxidizing agents and a reverted effect with expression of wild type XPC (D'Errico et al., 2006). Moreover they showed that XPC protects human skin cells from the killing effects of oxidants such as potassium bromate or those induced by X-rays and provided evidence that XPC is involved in the repair of 8,5'-cyclopurine 2'-deoxynucleosides and major oxidized DNA bases such as 8-oxoG (D'Errico et al., 2006).

Additionally, a study performed on mouse embryonic fibroblasts (MEFs) derived from *Xpc* KO mice and exposed to oxygen showed higher sensitivity, in terms of survival and mutation accumulation, than MEFs derived from *Xpa* KO or wild type mice (Melis et al., 2008a). Increased levels of oxidative stress upregulate NER factors including XPC and also links glutathione anti-oxidant response with NER regulation. Exposing XPC-silenced human glioma cells to arsenic trioxide diminished their anti-oxidant status thereby increased arsenic susceptibility and arsenic-induced cell death but did not affect the repair of the arsenic-induced DNA damage noting that arsenic exerts its cytotoxicity via the generation of ROS and inhibition of DNA repair (Liu et al., 2010).

Rezvani et al. documented that silencing of XPC in normal human keratinocytes lead to increased intracellular ROS levels, disturbed redox homeostasis, altered energetic metabolism and accumulation of mutations in the genomic and mitochondrial DNA which finally lead to tumorigenesis. Furthermore, they reported that at the molecular level, the deficiency of XPC triggered an increase in DNA-dependent protein kinase (DNA-PK) activity, which

subsequently activated AKT1 and NADPH oxidase 1 (NOX1) resulting in ROS production and accumulation of specific deletions in mitochondrial DNA (Rezvani et al., 2011a).

b. *In vivo* studies

In vivo experiments performed on C57BL/6J *Xpc* KO mice, in comparison to their wild type controls and *Xpa* mice, showed a strong exclusive increase in mutational load in the lungs of the *Xpc* KO mice during aging (Melis et al., 2008a). This result was explained by that XPC defect lead to lack of an adequate oxidative stress response, which lead to accumulation of mutations in the lungs especially that these organs are known to be constantly exposed to oxygen. No such increase in mutational load was found in wild type and *Xpa*-deficient mice. Since *Xpa*-deficient mice seemed unaffected in lung tumor incidence and mutational load, these results pointed toward an additional function of XPC in mice in response to oxidative DNA damage.

This finding was further supported by a 39-week exposure study through the diet with *XPC*- and *Xpa*-deficient (C57BL/6J) mice to the oxidative stressors diethylhexyl phthalate (DEHP) and paraquat which showed mice a significant increase in mutational load in the liver in *Xpc* KO mice when compared to wild type and *Xpa* mice (Melis et al., 2011). In addition, recent discoveries showed an absence of anti-oxidant (glutathione) response in *Xpc* KO mice upon exposure to the pro-oxidant DEHP while in wild type and *Xpa* mice it remained fully active (Langie et al., 2007; Melis et al., 2011). In addition, gene expression profiling of mutated liver tissue of *Xpc* KO mice showed a decreased anti-oxidant response and an enhancement of cell cycle progression (Melis et al., 2011). Another short-term *in vivo* study involved a two-week exposure of *Xpc* KO mice to equine estrogen demonstrated a non-significant increase in 8-hydroxyguanosine in liver but did not lead to enhanced sensitivity to 8-oxodG DNA adduct as compared to wild type mice (Okamoto et al., 2008).

Moreover, lymphocytes from *Xpc* KO mice showed accumulation of spontaneous lesions in the hypoxanthine guanine phospho-ribosyl transferase (HPRT) gene, which result from oxidative processes, suggesting a causative role for ROS (Wijnhoven et al., 2000). An interesting study was performed to elucidate the effect of combined XPC deficiency and *Apex* gene haploinsufficiency showing an increased predisposition to skin cancer after UV-B exposure. And since *Apex* gene is responsible for the activation of many transcription factors (including *Trp53*) by both redox-dependent and redox-independent mechanisms, its defect lead to imbalance in redox homeostasis which lead to alteration in cancer susceptibility (Meira et al., 2001a).

Another evidence that XPC is implicated in oxidative DNA damage and therefore in metabolic processes in the cell resides in the occurring incidences of internal tumors knowing that internal tissues are not exposed to UV light and are exposed rarely to chemicals inducing bulky adducts. For example, XPC defect has shown to be implicated in lung cancer (Wu et al., 2010). However, lungs are exposed to high levels of oxidative stress. Another example is that XPC defect has shown to be implicated in bladder cancer (Chen et al., 2007). In this context, the levels of XPC protein were found to be reduced in majority of bladder cancer patients (Chen et al., 2007; Xu et al., 2011; Yang et al., 2010). However, urinary bladder cells also come into contact with harmful (environmental) agents for extended periods of time through urine, which can cause DNA damage. Also, increased oxidative stress levels have been implied in colorectal, bladder and lung carcinogenesis (Chang et al., 2008; Romanenko et al., 2000; Salim et al., 2008; Seril et al., 2003; Soini et al., 2011; Sung et al., 2011). Furthermore, internal and skin tumors of XP-C patients have been compared in the past and results indicated a different mutation spectrum for internal tumors compared to skin tumors which were believed to result from unrepaired lesions caused by oxidative damage (Giglia et al., 1998).

4. Cell cycle control

Besides the removal of DNA lesions, evidence exists that XPC is implicated in cell cycle checkpoints regulation and apoptosis (Stout et al., 2005; Wang et al., 2004). These checkpoint controls intervene with proper DNA repair, apoptosis and cell cycle arrest and thereby are indispensable for maintenance of genomic stability and prevention of cancer. The first evidence for XPC potential role in cell cycle control is that during damage recognition the XPC–HR23B complex associates also with CETN2 a protein that stabilizes XPC and that is involved in centrosome duplication, indicating a direct link between GGR and cell division (Araki et al., 2001).

Several studies have implicated NER factors, including XPC, in ATM and ATR signaling and regulation. ATM and ATR protein kinases are key regulators in DNA damage response especially DSBs by interacting with cell cycle checkpoints leading to G1 or G2 arrest or S phase delay. ATM and ATR are able to phosphorylate many cellular substrates, for example the tumor suppressor protein p53. It was later shown that XPC was required for the association of ATM to the genomic DNA. Also, recent study showed that, upon UV radiation, SNF5, a member of SNF5/INI1 chromatin remodeling component, colocalizes and interacts

with XPC which facilitates the access of ATM to the damage site and thereby promoting NER. Furthermore, ATR appeared to be required for GGR, exclusively in S phase of human cells.

Several other studies revealed interactions between XPC and the tumor suppressor and cell cycle mediator p53 that is a key player in DNA damage-induced checkpoints and apoptosis. In humans, p53 positively regulates the expression of XPC and DDB2. The constitutive level of expression of XPC is controlled by p53, and can be induced by UV-light irradiation, enhancing GGR (Ford and Hanawalt, 1997). Hence, compromised p53 function might reduce GGR activity thereby facilitating accumulation of mutations and carcinogenesis. Additionally, XPC deficiency is strongly correlated with p53 mutations and malignancy observed in bladder tumors. Interestingly, a mutational hotspot at a nondipyrimidinic CpG site in codon 122 was detected in Trp53 of UVB-induced skin tumors in *Xpc* KO mice whereas not detected in *Xpa* KO and *Csa* KO mice.

On the other hand, XPC modulates activity of p53 at post-transcriptional level inhibiting its degradation by provoking formation of hHR23B-p53 complex, thereby preventing p53 degradation. Moreover, under cisplatin treatment, the p53 response was reduced due to XPC deficiency. XPC was suggested to play a critical role in initiating the signal transduction process after cisplatin induced DNA damage resulting in p53 activation and cell cycle arrest.

5. Oxidative DNA damage repair

Several studies discussed earlier showed increase in oxidative damage due to XPC deficiency. Two possible explanations were proposed. The first one is that NER repair oxidative damage scarcely in addition to its general known function in repairing bulky DNA adducts and helix-distorting damage. Supporting evidence to this option was that sometime oxidative DNA lesions lead to helix-distorting structures that trigger induction of NER pathway. Another evidence is that certain non-bulky lesions, like 8-oxoG and thymine glycol, are able to arrest partially RNA polymerase II during transcription leading to initiate NER. However, competition between BER and TCR for 8-oxoG repairs has been proposed. An important example is that XPC-deficient cells after exposure to oxidative stress accumulate 8,5'-Cyclopurine-2'-deoxynucleosides that represent a particular class of endogenous oxidative DNA lesions which are capable of blocking RNA pol II transcription and are repaired by NER. More recent studies furthermore indicate that intrastrand crosslink lesions such as G[8-

5]C, G[8-5m]mC, and G[8-5m]T can be caused by oxidative DNA damage and are a substrate for NER (Gu et al., 2006; Wang et al., 2012a).

The second explanation is that XPC is implicated in BER pathway. First of all, *Xpc* KO and *Xpa* KO mice showed differences in oxidative DNA damage sensitivity indicating that XPC has additional functions outside of NER. Since BER is the primary pathway for repairing oxidative lesions and XPC defect leads to increased sensitivity towards oxidative DNA damage, it is thought that XPC is implicated in BER pathway. It has been postulated that the XPC-hHR23B complex acts as a co-factor in the base excision repair of 8-hydroxyguanosine products by stimulating the activity of the BER DNA glycosylase OGG1 (D'Errico et al., 2006). This interaction was later confirmed by the investigation of specific XPC mutations. Analysis of the biochemical properties behind mutations in the *XPC* gene found in XP patients demonstrated a direct interaction between the N-terminal part that encompasses the P334 surrounding region of XPC and OGG1. The XPC/P334H mutation weakens the interaction with OGG1, resulting in a decreased capacity to regulate the glycosylase activity (Bernardes de Jesus et al., 2008). XPC is also able to interact with the repair factors thymine DNA glycosylase (TDG) and SMUG1, supporting the hypothesis that XPC might be involved in BER or G/T mismatch repair (Shimizu et al., 2003a). Also, the hHR23B factor was found to interact with BER protein 3-methyladenine DNA glycosylase (Kraemer et al., 1992). The XPC-hHR23B complex also recognizes 5R-thymine glycol (5R-Tg) lesions, which modulate BER. Recently, fibroblasts from different XP-C patients also showed impairment in base excision repair of oxidative DNA damage induced by methylene blue plus visible light (Kassam and Rainbow, 2007). Taking into account the multitude and diversity of presented interactions, either physical or regulatory, it is plausible to assume XPC is also involved in BER. XPC appears non-essential for BER, but might contribute to the effectiveness of this repair pathway by possibly recognizing or enabling recognition of oxidative lesions through signaling and regulatory functions.

D'Errico and colleagues have proposed a mechanism in which XPC–HR23B might bend DNA at sites of damage and, thus, facilitate loading and turnover of DNA glycosylases by direct protein–protein interaction or by competition with the DNA substrate (D'Errico et al., 2006). The possibly more supportive role of XPC in the removal or prevention of oxidative DNA damage proposed by several earlier groups is accentuated by the phenotype of XP-C patients. XP-C patients who are diagnosed early and are well protected from sunlight mostly show no evidence for any significant pathology that might indicate abnormal responses to oxidative damage, although primary internal tumors have been identified in two young XP-C patients (Giglia et al., 1998). In addition, one early-diagnosed XP-C patient (XP1M1), harboring the

(P334H) mutation, has been diagnosed with neuropathology. As mentioned earlier, evidence was provided that this P334H substitution can prevent stimulation of BER factor OGG1 (Bernardes de Jesus et al., 2008). Another XP-C patient (XP21BE) with neurological abnormalities has been identified, but neuropathology might be caused by another genetic defect other than impaired XPC functioning (Khan et al., 2009). Furthermore, potential allelic loss of *XPC* was observed in many of the human lung tumors (Hollander et al., 2005a). The late onset of adverse health effects due to lung tumors in *Xpc* KO mice suggests that lung or other internal tumors driven by oxidative DNA damage would be apparent only in older XP-C patients.

E. Therapeutic approach of XPC

Xeroderma pigmentosum group C (XP-C), known to be deficient in NER pathway, is a rare human syndrome characterized by hypersensitivity to UV light and a dramatic predisposition to skin neoplasms. Mutations within the *XPC* gene are by far the most common genetic alteration found in European and North African XP patients. To date, there is no curative treatment for XP-C patients and their cancer-free survival relies solely on full body protection from light and/or surgical resections of skin tumors. The harsh symptoms developed by XP-C patients urged scientists to develop several therapeutic strategies in an attempt to cure the disease. Early trials for treatment of XP-C patients constituted autologous grafts using UV sensitive cells which imposed only transient benefits (Ergün et al., 2002). Later, engraftment of patients with skin produced *ex vivo* with cells corrected for *XPC* mutation was a major advance. Warrick *et al.* used a retrovirus-based strategy to transduce the wild-type *XPC* gene into human primary XP-C keratinocyte stem cells and reconstitute their full NER capacity resulting in UV resistance (Warrick et al., 2012). Despite the successful genetic correction of *XPC* deficiency by the complementation strategy *in vivo* and in a relevant cell line, the approach implied potential adverse effects due to uncontrolled random integrations of the transgene, as such, undesirable effects have been reported in several complemented cells for disease treatment, especially in the hematopoietic system (Fischer and Cavazzana-Calvo, 2005; Hacein-Bey-Abina et al., 2003). In view of this result, genetically modified skin could lead to skin tumor development following engraftment. In addition, because of the ectopic expression of the *XPC* transgene, this strategy prevents physiological regulations of the *XPC* transcription, the importance of which has been described in other studies (Adimoolam and

Ford, 2002; Rezvani et al., 2007). Thus, an alternative and safer approach to curing XP-C defective cells is highly desirable.

Lately, Dupuy et al. provided an alternative and safer approach to cure XP-C defective cells (Dupuy et al., 2013). In their study, they described targeted correction of the Δ TG mutation in XP-C cells. In brief, Δ TG mutation, representing the deletion of a TG dinucleotide located in the middle of exon 9 that lead to the expression of an inactive and undetectable XPC truncated protein, is a founder mutation in North African patients and has been also described in almost 90% of Maghrebian XP-C patients (Soufir et al., 2010). The approach used in the study was nuclease-based targeted whose principle on the ability of engineered nucleases including meganuclease and TALE nucleases to generate a precise double-strand break at a specific locus and promote targeted homologous recombination (HR) with an exogenous DNA repair matrix (Daboussi et al., 2012; Urnov et al., 2010). They showed that demethylating treatment as well as the use of TALENTM insensitive to CpG methylation enables successful correction of the Δ TG mutation. Such genetic correction leads to re-expression of the full-length XPC protein and to the recovery of NER capacity, attested by UV-C resistance of the corrected cells. The full-length XPC protein was steadily expressed over time, demonstrating the stability of the genetic correction. Finally, NER rescue was confirmed by the cell survival rate following different UV exposures. However, the use of demethylation treatment, 5-aza-dC, imposed deleterious effect on the corrected cells which may represent a hurdle for its application in primary keratinocytes, the relevant primary cells for genetic correction (Dupuy et al., 2013).

Finally, the targeted nature of nuclease-assisted gene correction offers a major advantage compared to conventional gene therapy strategies relying on retrovirus-assisted gene complementation. Nuclease-assisted gene correction permits the functional gene to benefit from the natural chromosomal context and regulatory regions known to play key roles in the fine-tuning of gene expression. This is particularly true for the *XPC* promoter region, shown to contain regulatory elements located 1,700 bp upstream from the Transcription Start Site (TSS) (Xu et al., 2012). Likewise, gene therapy using nucleases enabled the full-length XPC protein to be re-expressed at a level compatible with normal UV-dependent DNA damage repair. Otherwise, overexpression of XPC could be detrimental due to its versatile capacity to recognize physiological distortions in the DNA double helix and to bind to DNA mismatch with high affinity as for example when XPC-GFP or HA-RAD4 were overexpressed in murine fibroblasts or in yeast respectively, a rapid degradation of these proteins by the proteasome was observed (Lommel et al., 2002; Ng et al., 2003). Finally, we observed a

steady expression of the protein which indicated that the expression of the corrected XPC was not down-regulated with time suggesting that targeted nuclease approaches are unlikely to trigger epigenetic silencing of the corrected gene, as reported in complementation using retroviral approaches (Gram et al., 1998).

VI. DNA repair in stem cells

Stem cells like every other cell in the body are exposed to DNA insults and damage. The genomic stability of SC is threatened by endogenous chemical and exogenous environmental insults (Sancar et al., 2004). Since the SC are responsible for the maintenance of tissues by self-renewal and differentiation capabilities, defect in repair of DNA damage can be transmitted to their differentiated daughter cells, thereby compromising tissue integrity and function. As a consequence, mutations that diminish the reconstitution potential of SC can result in atrophy of tissues and aging phenotypes, whereas mutations providing a selective advantage to the mutated cells can lead to carcinogenesis (Rossi et al., 2008). Therefore, a delicate balance must be struck to prevent exhaustion and transformation of the SC pool while maintaining the ability of SC to preserve homeostasis and to respond to injury when necessary.

The genomic integrity of SC is of high importance and for that reason SC are naturally protected by specific mechanisms which include localization to a specific microenvironment, resistance to apoptosis, limitation of ROS production, and maintenance in a quiescent state (Orford and Scadden, 2008; Rossi et al., 2008). In addition, SC decision to either self-renew or differentiate is balanced by complex network of cell-intrinsic regulations thereby serving as a control for the number and functional quality of SC. Furthermore, both the integrity and function of SC is maintained by the existence of DNA repair mechanisms (Kenyon and Gerson, 2007). Altogether, these attributes of SC ensure tissue maintenance and function throughout the lifetime of an organism, while limiting atrophy and cancer development (**Figure 21**).

DNA repair is crucial for SC integrity and function and several studies have showed that mutations in genes regulating DNA repair pathways lead to severe clinical consequences observed in both humans and mice. To address the role of DNA repair pathways in SC, it is crucial to shed light on important molecular players of these pathways.

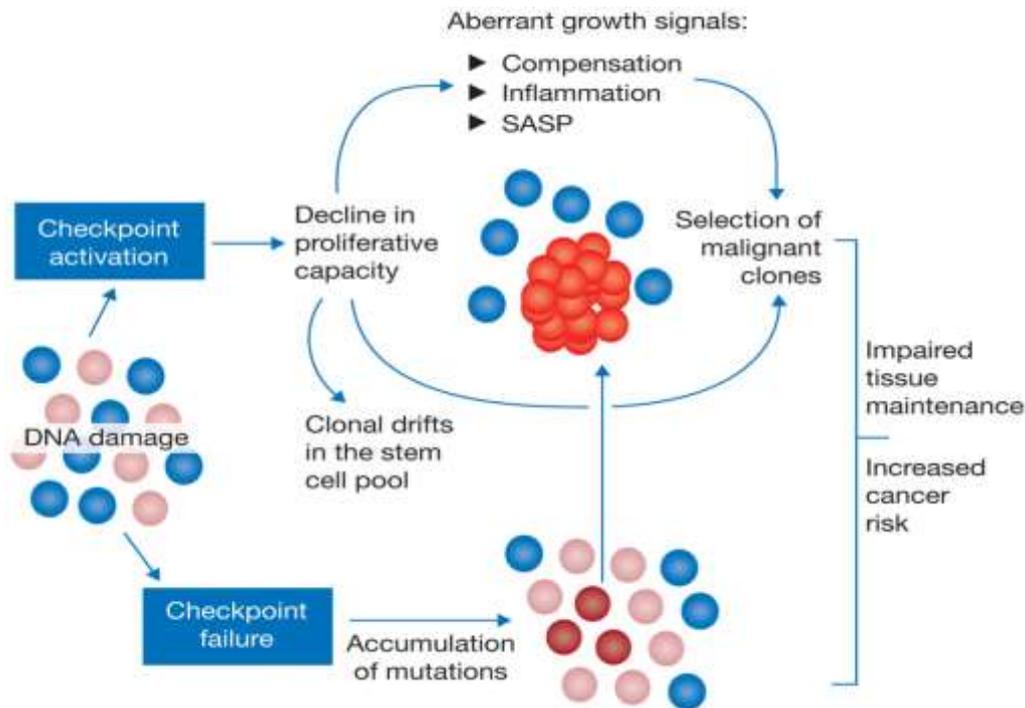


Figure 21. Accumulation of DNA damage on tissue stem cells during aging leads to impaired tissue maintenance and increased cancer risk. Accumulation of DNA damage associated with aging could either activate or not checkpoints that remove damaged stem cells by inducing apoptosis, cell cycle arrest or differentiation. Checkpoint activation can lead to clonal drifts or imbalances in the pool of remaining stem cells and impairment of proliferative capacity of growing stem cells that will in turn increase the selective pressure for the outgrowth of mutant cell clones. This process is further accelerated by aberrant growth signals originating from compensatory feedback loops to maintain tissue homeostasis, the accumulation of senescent cells exhibiting a secretory phenotype (SASP), or inflammation as a consequence of immune reactions targeting damaged cells. Checkpoint failure in response to DNA damage leads to increased risk of acquiring mutations that will lead to a selective growth advantage in the context of damage accumulation, checkpoint induction and growth inhibition in the pool of aging stem cells. *Figure adapted from (Behrens et al., 2014).*

A. DSB repair in SC

Several clues exist in the literature for the importance of HR and NHEJ pathway in repair of DSBs. For instance, the MRN complex constituting of MRE11, RAD50, and NBS1 is required for both HR and NHEJ pathway. It senses DSBs, unwinds the damaged region of DNA, serves as part of the repair scaffolding, and induces downstream signaling including ATM activation. Deletion of any component of the MRN complex results in embryonic lethality in mice (Hakem, 2008). However, mice bearing a hypomorphic Rad50^{k22m} mutation are viable but die around 2.5 months from of B cell lymphoma or bone marrow failure due, in part, to p53-dependent DDR-mediated apoptosis and loss of HSC function (Bender et al., 2002).

1. HR repair in SC

As for ATM (ataxia-telangiectasia mutated), known to be a DNA-damage-sensor, mutations in humans present blood vessel abnormalities, cerebellar degeneration, immunodeficiency, and increased risk of cancers (Hoeijmakers, 2009). In mice, *Atm*-deficiency leads to extreme sensitivity to gamma-irradiation exposure, decreased somatic growth, neurological abnormalities, decreased T cell numbers, premature hair graying and infertility (Barlow et al., 1996). Moreover, ATM's crucial role as a DDR component has been validated in various types of SC. In HSC, *Atm*-deficiency leads to increased ROS levels and display an overall decrease in number and function over time, leading to eventual hematopoietic failure (Ito et al., 2004a, 2006). In melanocyte SC, *Atm* deficiency sensitizes mice to IR-induced premature differentiation, resulting in hair graying (Inomata et al., 2009). In germ SC, *Atm*-deficiency in mice leads to alteration in germ cell development resulting in progressive loss in germ SC (spermatogonia) and infertility (Takubo et al., 2008).

Regarding ATR (ataxia-telangiectasia related), also known to be a DNA-damage-sensor, its mutations result in developmental defects in mice (pregastrulation lethality) and humans (Seckel syndrome) (Hakem, 2008; Hoeijmakers, 2009; Seita et al., 2010). Conditional deletion of *Atr* in adult mice leads to the rapid appearance of age-related phenotypes, such as hair graying, alopecia, kyphosis, osteoporosis, thymic involution, and fibrosis, which are associated with SC defects and exhaustion of tissue renewal and homeostatic capacity (Brown and Baltimore, 2000; Ruzankina et al., 2007).

Moreover, mutations in *BRCA1* and *BRCA2*, two DSB mediators that trigger DNA repair through the HR pathway, lead to a major increase in the risk of developing breast and ovarian cancers in women, which, at least in the breast, has recently been linked to the accumulation of genetically unstable mammary SC (Liu et al., 2008).

2. NHEJ repair in SC

As discussed previously, the NHEJ pathway is a complex interplay of molecules such as end-binding and end-processing proteins Ku70, Ku80, DNA-PKcs, and Artemis and ligation complexes XRCC4, LigIV, and Cerrunos (Lombard et al., 2005). In mice, inactivation of various NHEJ genes has demonstrated their essential function in lymphocyte development and prevention of lymphoma since NHEJ is critical for V(D)J recombination during

lymphocyte maturation. Mice lacking Ku70 and Ku80 displayed stress-induced HSC self-renewal defects associated with poor transplant ability, increased apoptosis, decreased proliferation, and impaired lineage differentiation (Kenyon and Gerson, 2007; Rossi et al., 2007a). Mice carrying a *Lig4*^{y288c} hypomorphic mutation showed severe HSC defects along with growth retardation, immunodeficiency, and pancytopenia (Kenyon and Gerson, 2007; Nijnik et al., 2007). In humans, until now, no spontaneous mutations in NHEJ pathway components have been reported in human syndromes associated with premature aging or carcinogenesis.

B. MMR repair in SC

Defective HSC activity has been found in mice lacking *Msh2*, an important factor in MMR pathway, along with enhanced microsatellite instability observed in their progeny (Reese et al., 2003). In addition, elevated frequencies of hematological, skin, and gastrointestinal tumors has been found in mice lacking *Msh2* and *Mlh1*, enforcing the critical role of the MMR in preventing accumulations of oncogenic mutations (Hakem, 2008). Lynch syndrome, a hereditary nonpolyposis human colorectal cancer, is induced by mutations in MMR pathway components and presents with about an 80% lifetime risk of developing colorectal cancers as well as other malignancies (Hoeijmakers, 2009).

C. NER repair in SC

In mice, *Ercc1* deficiency, a component of both NER and intrastrand crosslink (ICL) repair, is lethal where it leads to death within 4 weeks of birth and presents senescence of HSC, depletion of its progenitors leading to multilineage hematopoietic cytopenia, and a defective response to DNA crosslinking by mitomycin C (Hasty et al., 2003; Prasher et al., 2005). In mice lacking XPD, an essential NER component, decreased HSC function with reduced self-renewal potential and increased apoptosis levels has been found (Rossi et al., 2007a).

VII. DNA repair in HSC

HSC are one of the best studied adult stem cells in which they produce all blood cell lineages (Orkin and Zon, 2008). HSC are continuously exposed to DNA damage threatening their genomic stability which renders DNA repair of vital importance to maintain the hematopoiesis (Figure 22).

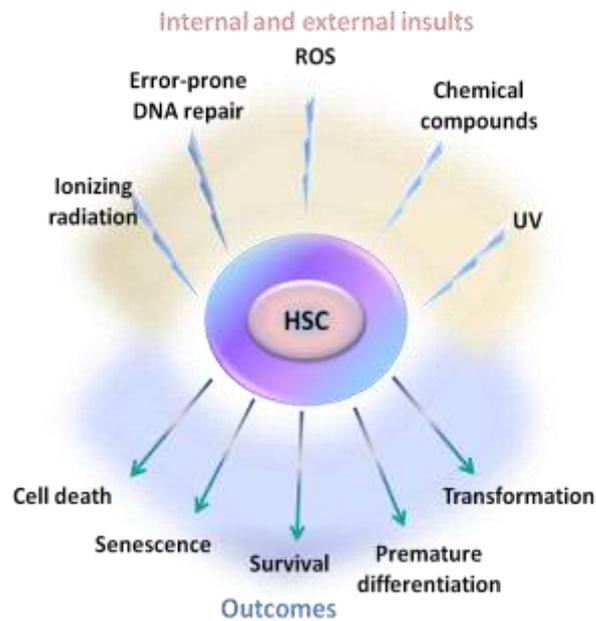


Figure 22. HSC genomic integrity threatened by several insults and protected by several stress responses. Figure designed based on information from (Suda et al., 2011).

Several investigations have been held to study the response of HSC to DNA damage especially DSB. A major breakthrough in this domain of research has been carried on by Mohrin et al. and Milyavsky et al. who investigated the response of mouse and human HSC to ionizing radiation (IR) respectively.

On one hand, Mohrin et al. has showed that, upon exposure to IR, quiescent HSC use NHEJ repair which is associated with acquisition of genomic rearrangements while proliferating HSC use HR which is error-free. Mohrin et al. has also showed that HSPC are intrinsically more radioresistant to IR than myeloid progenitors such that, under DNA damage, HSC undergo senescence while myeloid progenitors often undergo apoptosis. In accordance, Meijne et al. has previously reported that HSC are more radioresistant than their downstream progeny (Meijne et al., 1991). It is postulated that the strong p53-mediated induction of *p21* coupled with high basal level of pro-survival genes in HSPC protect them against killing effects of increased pro-apoptotic gene expression, resulting mainly in growth arrest. In addition, Mohrin et al. has found that it restricts the ability of HSC to use HR and force them

to use error-prone NHEJ mechanism to repair DSBs which make them prone to acquire cytogenetic aberrations than can contribute to increased malignancy risk (Mohrin et al., 2010). On the other hand, Milyavsky et al. has worked on the DNA damage response in human HSC isolated from cord blood which differs from more mature hematopoietic progenitors. He showed that human HSC are more sensitive to the cytotoxic effects of IR compared to committed progenitors. He also showed that the primitive human HSPC exhibited delayed DSB rejoining and persistent DDR foci and underwent higher levels of p53/ASPP1-dependent apoptosis compared to progenitor population in response to IR (Milyavsky et al., 2010). Therefore, according to studies of Mohrin et al. and Milyavsky et al., Blanpain et al. has suggested that irradiated UCB-derived HSC were efficiently eliminated since they could be detrimental to the organism due to its reproductive purpose while irradiated mouse adult HSC survived and exhibited efficient DNA repair of irradiated mouse adult HSC which allows the protection of the most important cells of the tissue even if this occurs at a cost for their long-term genomic integrity in many cases (**Figure 23**).

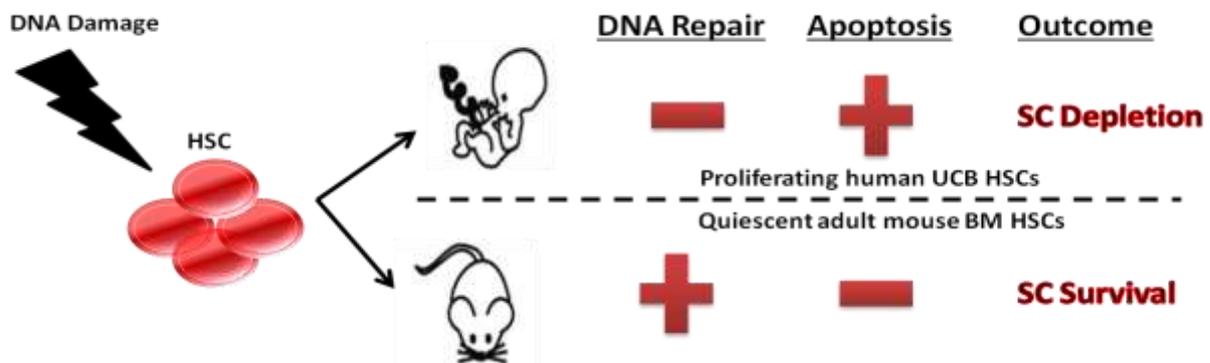


Figure 23. DNA-Damage Response in human (fetal) versus mouse (adult) HSC. Human UCB-derived HSC and mouse BM-derived HSC possess opposite outcomes following irradiation-induced DNA damage along with different consequences for their overall maintenance and genomic integrity. *Figure designed based on information from* (Blanpain et al., 2011).

In congruence to the results obtained by Mohrin and Milyavsky and their colleagues, Shao et al. has used a highly sensitive qPCR-based cell free *in vitro* NHEJ assay to show that human BM HSCs (CD34⁺CD38⁻cells) are less proficient in the repair of DSBs by NHEJ than HPCs (CD34⁺CD38⁺cells). In contrast, mouse quiescent HSCs (Pyronin-Y^{low} LKS⁺cells) and cycling HSCs (Pyronin-Y^{hi} LKS⁺cells) repaired the damage more efficiently than cycling HPCs (LKS⁻cells). Consistently, both cycling and quiescent mouse BM HSC expressed significantly great levels of KU70, KU80, ligase 4 and XRCC4 mRNA than HPCs.

Nevertheless, nuclear protein extracts from quiescent mouse BM HSC had about two-fold greater NHEJ activity than those from cycling mouse BM HSCs which is in agreement with Mohrin et al. who showed that quiescent mouse BM HSC exhibited a higher NHEJ activity than the cycling HSC. Thus, the difference in the abilities of human and mouse HSC and HPC to repair DSB through NHEJ is likely attributed to their differential expression of key NHEJ DNA damage repair genes such as *LIG4* where human progenitors express higher levels of ligase 4 mRNA than HSC (Shao et al., 2012).

Other studies have shed light on the importance of niche factors such as cytokines and environmental signals in the repair of DNA damage by HSC. De Laval et al. has demonstrated for the first time that thrombopoietin (TPO) cytokine and its Mpl receptor involved in HSC maintenance can also regulate the response to DNA damage by controlling their DSB machinery. De Laval et al. has found that loss of Mpl increases γ -irradiation-induced genomic instability in HSPCs and they showed that TPO stimulates DNA repair in vivo and in vitro by increasing DNA-PK activity and NHEJ-mediated repair efficiency in HSPCs which ensures the chromosomal integrity of HSC and limits their long-term injury in response to IR. They also showed that in absence of TPO, the DNA repair is defective in irradiated wild-type and *Mpl* KO mouse or human HSPC (de Laval et al., 2013).

Interestingly, different doses of IR alter the HSC function and recovery. Simonnet et al. have examined the effect of IR exposure using different doses on HSPC over time covering both BM suppression and recovery. The LSK cell frequency and BM cellularity were decreased in dose-dependent manner. Shortly after IR exposure, they have identified a radioresistant subset of HSC that belonged to SP fraction with transient phenotypic changes at their surface including an increase in the Sca-1 expression level and a dramatic decrease in that of c-Kit both in a dose-dependent manner. In addition, IR has driven the cells from quiescent to cycling state. Ten weeks of IR exposure, a phenotypic imbalance within LSK stem/progenitor cell compartment was observed which accounted for long-lasting hematopoietic injury and which resembled the phenotypic changes during aging phenomenon. The proportion of CD150⁻Flk-2⁺ cells noted as MPP was dramatically decreased for irradiated mice 10 weeks after IR exposure; this reduction was accompanied by an increase in the LSK frequency of CD150⁺Flk-2⁻ denoted as LT-HSC and increase in ST-HSC purified as CD150⁻Flk2⁻ cells. The CD150⁺Flk2⁻ cells showed impaired reconstituting ability, an increased tendency for apoptosis, and accrued DNA damage accumulation. In addition, when HSC from nonirradiated donors were transplanted into irradiated but not in control recipients they were able to engraft and contribute to host hematopoiesis (Simonnet et al., 2009).

A. DDR of HSC

DDR differs between mouse and human HSC. Mouse HSC showed decreased sensitivity to cytotoxic agents, such as 5-FU. The DDR response of quiescent mouse HSC is biased to the prosurvival outcome due to p53-mediated activation of double-strand break (DSB) repair (Mohrin et al., 2010). While protecting HSC from environmental insults, this mechanism leads to the accumulation of DNA damage in the pool of multipotent cells, increasing the chance of leukemic transformation. By contrast, human HSC are actually sensitized to apoptosis after irradiation, sacrificing damaged HSC in favor of maintaining genomic integrity (Milyavsky et al., 2010). This DDR is also p53 dependent, since loss of p53 compromises long-term HSC function and results in persistent DSB. Thus, an attractive hypothesis is that humans and mice have evolved different strategies to deal with DNA damage, reflecting different lifespan and age to reproductive maturity (**Table 4**).

Damage Repair Pathway	Studied Gene	Mouse model	Effect on HSC maintenance	References
HRR	ATM	Atm ^{-/-}	Mice older than 24 weeks showed progressive bone marrow failure resulting from a defect in HSC function, with a decreased stem cell number, loss of repopulation capacity, loss of lymphoid function, increased apoptosis that was associated with elevated reactive oxygen species which was corrected by treatment with anti-oxidative agents.	(Ito et al., 2004b)
	Rad50	Rad50 ^{s/s}	Mice died with complete bone marrow depletion as a result of progressive hematopoietic stem cell failure and chromosomal instability.	(Bender et al., 2002)
NHEJ	Ku70	Ku70 ^{-/-}	Loss of HSC quiescence and maintenance; rescued by Bcl2 overexpression.	(Qing et al., 2014)
	Ku80	Ku80 ^{-/-}	Attenuation of HSC self renewal and functional exhaustion.	(Rossi et al., 2007b)
	DNA-PKcs	PKcs ^{-/-}	T and B lymphocyte development was arrested and V(D)J coding-end rearrangement was deficient, but V(D)J signal-end joining ability was intact. NO growth retardation or T cell lymphoma was detected.	(Kurimasa et al., 1999)
		PKcs ^{3A/3A}	Dephosphorylation DNA-PKcs in this model leads to premature death of mice due to congenital bone marrow failure, impaired proliferation of HSC.	(Zhang et al., 2011b)
	Lig1	Lig1 ^{-/-}	Embryonic death due to failure in fetal liver erythropoiesis. Fetal liver cells were unable to repopulate lethally irradiated recipients resulting in bone marrow failure by 10 weeks. Mice that survive exhibit anemia, enlarged erythrocytes and reticulocytosis.	(Bentley et al., 2002)
	Lig4	Lig4 ^{4288C}	Increased proliferation, impaired maintenance, progressive loss during aging.	(Nijnik et al., 2007)
MMR	Msh2	Msh2 ^{-/-}	Hematopoietic serial repopulation defect and stem cell exhaustion because of accumulation of genomic microsatellite instability in CFU and LSK clones.	(Reese et al., 2003)
NER	XPA	XPA ^{-/-}	Significant decrease in CFU-GM colonies only in aged mice (57 wks) but no difference in PB value or BM count.	(Prasher et al., 2005)
	XPD	XPD ^{TTD}	Symptoms similar to the human disease. Upon competitive transplantation, HSC show repopulation defect with reductions in whole-bone marrow engraftment of B cell, T cell, myeloid and granulocytes.	(Rossi et al., 2007b)
NER and ICL repair	ERCC1 (XPF)	ERCC1 ^{-/-}	Multilineage cytopenia, fatty replacement of BM, significant reduction in proliferative reserve of hematopoietic progenitors and stress erythropoiesis, effect manifested with aging.	(Prasher et al., 2005)
BER	PARP1	PARP1 ^{-/-}	No spontaneous defect in hematopoiesis; under oxidative stress, repopulating capacity of HSC is compromised in transplanted recipient mice and effect corrected by salidroside stimulation.	(Li et al., 2014)
Telomere maintenance	MTer	G3mTer ^{-/-}	Limited hematopoietic reserve and defective serial repopulation ability, effect rescued by p21.	(Rossi et al., 2007b)
ICL repair	FancC	FancC ^{-/-}	7-fold to 12-fold reduced in vivo repopulating ability of pluripotent hematopoietic stem cells compared with FancC ^{+/+} cells.	(Haneline et al., 1999)
	Fancd1 (BRCA2)	Brca2 ^{Δ27/Δ27}	Low numbers of CFCs either young or adult, a high incidence of spontaneous chromosomal instability, a very severe hematopoietic syndrome upon irradiation, marked repopulation defect, very significant proliferation defect.	(Navarro et al., 2006)
	Fancd2	Fancd2 ^{-/-}	Reduced HSC content and hematopoietic activity in BM, defective long-term in vivo repopulating ability.	(Parmar et al., 2010)
	FancG	Fancg ^{-/-}	Reduction in hematopoietic progenitor/stem cell compartment, failure in LT and ST reconstitution, defect in quiescence, migration in response to CXCL12 and in BM homing	(Barroca et al., 2012)
HRR, BER, NER	P53	P53 ^{+/-}	Higher numbers of proliferating hematopoietic stem and progenitor cells in old age compared with aged wild-type mice.	(Dumble et al., 2007)
		P53 ^{+tm}	Phenotypes of premature aging. Many aged p53 ^{+tm} organs exhibit reduced cellularity and atrophy, suggesting defects in stem-cell regenerative capacity. HSC numbers and proliferative potential from old mice fail to increase with age, reduced engraftment during transplantation unlike those of their p53 ^{+/-} and p53 ^{+/-} counterparts.	

Table 4. Overview of hematopoietic defects detected in mouse models of genomic instability disorders bearing deficient DNA repair pathways. As a note, all mice models described in this table exhibit hypomorphic mutations except for p53^{+tm} which exhibit a hypermorphic mutation.

VIII. DNA repair in leukemic CSC

Maintenance of HSC by balance of its self-renewal and differentiation properties is indispensable for homeostasis of body. Any deregulation in HSC function or acquisition of self-renewal capabilities by mature progenitor cells could lead to leukemias which represent the cancers of the blood system (Passegué, 2005). Interestingly, human leukemia is believed to contain stem cells that are characterized by aberrant self-renewal and differentiation capacities and have a variable developmental origin (Clarke and Fuller, 2006; Jordan et al., 2006). The leukemic stem cells (LSC) are the major cause for the persistence of the disease, its resistance to cancer therapy, and cancer relapse (Elrick et al., 2005; Jordan et al., 2006). LSC exist in acute myeloid leukemia (AML) and chronic myelogenous leukemia (CML). LSC maintain the same protective mechanisms as normal HSC such as quiescent cell-cycle status, localization to a hypoxic niche, and DDR mechanisms that allow them to escape cancer therapy (Guzman and Jordan, 2009). In this context, LSC in both CML and AML have been found to be quiescent (Elrick et al., 2005; Guan et al., 2003; Ishikawa et al., 2007). Moreover, it has been demonstrated that genetic alterations that cause AML such as FLT3/ITD or that cause CML such as BCR-ABL result in elevated ROS production which drives genomic instability leading to DSB formation and altered repair that can lead to acquisition of genomic changes. It has also been reported that leukemic cells are often associated with defects in NHEJ which leads to upregulation of A-NHEJ pathway that can create chromosomal deletions and translocations (Sallmyr et al., 2008a).

A. Genomic instability of CML

CML is a two-stage blood disease caused by the acquisition of the chromosomal translocation fusion product BCR/ABL in HSC. BCR/ABL expression increases intracellular ROS levels, which in turn enhances oxidative stress and DNA damage and deregulates DNA repair mechanisms leading to mutations and chromosomal aberrations (Perrotti et al., 2010). For instance, cells expressing BCR/ABL have unfunctional MMR, mutagenic NER, and compromised DSB repair (both HR and NHEJ) (Burke and Carroll, 2010; Deutsch et al., 2001; Slupianek et al., 2002, 2006). BCR/ABL inhibits apoptosis upon DNA damage, thereby allowing their survival (Burke and Carroll, 2010; Deutsch et al., 2001; Slupianek et al., 2002, 2006). Therefore, genomic instability induced by BCR/ABL has major implications for the pathogenesis and treatment of CML since it can facilitate disease progression from chronic to acute phase and promote the acquisition of resistance against the current drugs used to treat

CML tyrosine kinase inhibitors such as imatinib. A recent study by Sallmyr et al. has showed that BCR-ABL-positive CML cells exhibit downregulation of Artemis and DNA ligase IV which are key proteins in NHEJ pathway and exhibit an upregulation of DNA ligase III α and the protein deleted in Werner syndrome WRN leading to increased activity of A-NHEJ which is thought to ensure survival of AML cells but also to increase genomic instability and drive disease progression (Sallmyr et al., 2008b).

B. Genomic instability in AML

Human acute myeloid leukemia (AML), the most common leukemia type in adults, arises from neoplastic transformation in bone marrow cells. Based on the stage of differentiation that pluripotent stem cells have reached at the time of diagnosis, the traditional French–American–British classification (FAB) further categorized the heterogeneous AML into subtypes; e.g., along the monocyte-macrophage lineage, myeloblastic (M1, immature; M2, mature), promyelocytic (M3), myelomonocytic (M4) and monocytic (M5) leukemia subtypes were proposed based on morphological and cytochemical criteria (Bennett et al., 1976). AML cell lines corresponding to these subtypes have been established since late 1970s.

Several studies have related AML to perturbed DNA damage. For instance, Fan et al. have showed that internal tandem duplication (ITD) mutations of the FMS-like tyrosine kinase-3 (FLT3) receptor which is found in AML patients have decreased capacity for NHEJ and increased capacity for A-NHEJ resulting in high frequency of DNA deletions (Fan et al., 2010). In this context, in a study on AML SC, expression of PML/RAR or AML1/ETO fusion oncoproteins in murine HSC induced high levels of DNA damage and activated a *p21* dependent cell-cycle arrest, which allowed them to repair excessive DNA damage and to escape apoptosis, thereby maintaining their leukemic self-renewal capacity and providing a strong selective advantage (Viale et al., 2009). In the absence of *p21*, AML SC were more sensitive to replicative and therapeutic stress, and *p21* null HSC expressing PML/RAR or AML1/ETO were unable to transplant the disease into recipient mice, indicating a failure to maintain LSC activity (Viale et al., 2009).

IX. Role of NER in HSC maintenance

In order to elucidate the role of NER pathway in maintenance of HSC, *Rossi et al.* performed a study examining HSC reserves and function with age in mice deficient in NER. Deficiency in NER genomic maintenance pathways was generated by using XPD knockout mice defective in NER of helix-distorting DNA lesions. This study revealed fundamental results. Indeed, the frequency of LT-HSC increased significantly with age in XPD-deficient mouse models, identical to what is observed in wild type mice whereas the frequency of the hematopoietic progenitor cells decreased. This demonstrated that defects in NER mechanism are not essential for establishing or maintaining the relatively quiescent LT-HSC but are indispensable for maintenance of the more proliferative progenitor lineages. Thus DNA repair is not required for maintenance of HSC number. On the other hand, although deficiencies in NER pathway did not deplete stem cell reserves with age, stem cell functional capacity was severely affected under conditions of stress, leading to the loss of reconstitution and proliferative potential, diminished self-renewal, increased apoptosis and, ultimately, functional exhaustion. Thus DNA repair is required for maintenance of HSC function. Furthermore, diminished ability of HSC to proliferate and self-renew in DNA repair-deficient mice that were not exposed to exogenous genotoxic stress revealed that age-dependent spontaneous or endogenous DNA damage accumulation is responsible for the loss of HSC function (*Rossi et al., 2007a*).

Therefore the genome of quiescent HSC is susceptible to spontaneous DNA damage. In fact, more DNA damage is detected in HSC than progenitor cells isolated from aged mice. This could imply that progenitor cells are more prone to apoptosis or that they have a greater capacity for DNA repair relative to HSC. Regardless, the net result is that DNA damage accumulates in quiescent cells, while causing attrition of proliferating cell. Thus the bone marrow of aged organisms is hypocellular, enriched for LT-HSC and has diminished reconstituting capacity (*Niedernhofer, 2008a*).

Recently, study by *Beerman et al.* on DNA damage repair in quiescent/cycling and young/aged HSC has revealed that quiescent HSC appear to accumulate DNA damage during aging which is repaired upon entry into cell cycle. The approach they used to detect DNA damage is comet assays. In addition, using microarray expression analysis, they found that quiescent HSC have attenuated expression of DNA damage repair and response genes and they found that the NER pathway is remarkably downregulated. Surprisingly, they have found that the entry of the HSC into cell cycle is accompanied by upregulation of the DNA repair pathways which allows the repair of strand breaks and in consequence allows the maintenance of HSC and its sustained survival. Therefore, the novel results published validate that HSC are

not geno-protected during aging and instead show that quiescence of HSC attenuates DNA repair leading to accumulation of DNA damage with aging (Beerman et al., 2014) (**Figure 24**).

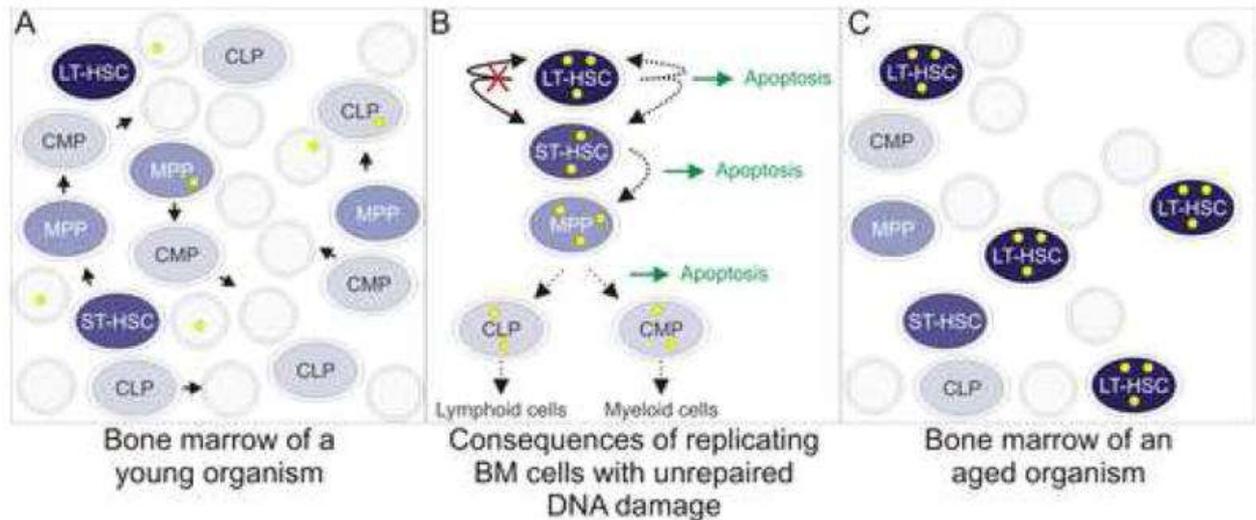


Figure 24. Model of how hematopoietic stem cell function is lost as a consequence of unrepaired DNA damage. (A) The bone marrow of young mammals contains relatively rare, quiescent long term reconstituting hematopoietic stem cells (LT-HSC), multi-potent progenitors (ST-HSC and MPPs) and more committed progenitors (CLPs and CMPs) as well as abundant terminally differentiated lymphoid and myeloid cells (unlabeled). Progenitors proliferate to produce more committed cells (arrows). DNA damage accumulates in the nuclei of cells with time (yellow dots). (B) As differentiated cells need replacing, HSC are recruited to proliferate. However, if DNA damage has accumulated in LT-HSC, this impairs their function, preventing self-renewal and proliferation (red cross) or triggering apoptosis (green arrow). More committed cells that have accumulated genome damage are prone to apoptosis (green arrows) leading to depletion of these cell populations. (C) The net result is hypocellularity and a relative enrichment of LT-HSC harboring DNA damage in the bone marrow of aged organisms.

Figure adapted from (Niedernhofer, 2008).

X. Role of NER in leukemia

Hsu and his colleagues systematically examine NER in a range of acute myeloid leukemia cell lines. In their study, they selected four human AML cell lines representative of various stages of differentiation on the myelo-monocytic pathway: KG-1a, KG-1, HL-60 and THP-1. In brief, KG-1 is a myeloblastic leukemia where it is blocked in cell maturation at the myeloblast stage (M2) (Koeffler and Golde, 1978). KG-1a is a less mature subclone of KG-1 identified as promyeloblastic leukemia (Koeffler et al., 1980). HL-60 is a promyelocytic leukemia (M3) (Rovera et al., 1979) which exists as several sub-lines due to its genomic instability (Fisher et al., 1997). THP-1 is a monocytic leukemia (M5). KG-1, HL-60 and THP-1 but not KG-1a can be differentiated into macrophage-like cells with TPA.

In this study, NER has been measured in several human acute myeloid leukemia cell lines, before and after differentiation into macrophage-like cells. Regarding, UV induced lesions, CPDs were not proficiently repaired in AML cell lines but (6-4)PPs were rapidly removed without any significant difference between naive and differentiated cells in both cases. This result for leukemia cell line is in congruence with what was previously observed with primary human neurons (Nouspikel and Hanawalt, 2002) but is in contrast to what was observed with NT2 neuroteratoma cells, which displayed a markedly slower repair of (6-4)PPs upon differentiation into neuron-like cells (Nouspikel and Hanawalt, 2000b). Regarding cisplatin-induced chemical intra-strand crosslinks, the various cell types behaved quite differently in this assay, with HL-60 and THP-1 being the most repair-proficient and their differentiated cells showed a marked repair deficiency. On the contrary, the less differentiated cells KG-1 and KG-1a lower NER activity than HL-60 and THP-1 and were less efficient at removing cisplatin adducts than were their more differentiated counterparts. However, the unexpected result in KG-1 and its subclone KG-1a may be the result of the carcinogenic transformation, rather than a consequence of the regular process of differentiation. In addition, all cell lines, whether differentiated or not, proficiently repaired CPDs in both transcribed and non-transcribed strands, but with a faster kinetics in the transcribed strand showing that leukemic cells are all TCR-proficient and that they exhibited DAR phenomenon as neuron-like cells (Nouspikel and Hanawalt, 2000b). Furthermore, the analysis of NER gene expression in these cell lines at the transcriptional level did not show any difference in expression of NER-related genes between the cell types with the exception of a higher expression of CSB in differentiated HL-60 cells. Interestingly, p48 subunit of DDB, encoded by the *XPE/DDB2* gene, was strongly reduced upon differentiation of HL-60 and THP-1 but not KG-1. Thereby, knowing that p48, which is crucial in the recognition of DNA distortions generated by CPDs,

is up-regulated after UV irradiation in a p53-dependent manner (Hwang et al., 1999) and knowing that in mammalian cells, the transactivating role of p53 has been shown to be crucial to the efficient GGR of CPDs in vivo (Ford and Hanawalt, 1995, 1997), and knowing that most AML cell lines have defective p53 status, Hsu and his colleagues postulated that the unfunctional p53 in leukemic cell lines imply low p48 levels leading to lower CPD damage recognition which might be the reason for that myeloid leukemia cell lines exhibit very little repair of CPDs (Hsu et al., 2007).

XI. XPC function in hematopoietic cells

A. XPC function in BM cells

The studies of XPC represent good models for GGR separate from TCR (Friedberg et al., 2006). As mentioned earlier, in humans, XPC deficiency leads to XP cancer-prone disease due to defective NER. XPC patients develop skin cancers prior to age 20 and develop internal cancers with aging. One study showed myelosuppression in an XPC patient (Salob et al., 1992). However, it is postulated that human XPC patients could exhibit myelosuppression if they lived to advanced age. Fischer and his colleagues were the first to examine the BM of an *Xpc* KO model (B6;129s7-*XPC*^{tml/Brd} mice) and to study to effect of XPC deficiency on response to carboplatin which, like cisplatin, produce a DNA damage spectrum, mainly 1,2 and 1,3 platinum diadducts that are substrates for NER *in vitro* (Shivji et al., 2006; Trego and Turchi, 2006). First of all, they found that XPC contributed substantially to cell survival since 60% of *Xpc* KO mice died whereas no death was reported for the wild-type mice upon carboplatin treatment. They also found that BM of *Xpc* KO mice exhibited 10-fold greater sensitivity to carboplatin compared to strain-matched wild-type bone marrow. In addition, the effect of XPC on BM cell survival was contributed to its impact on cell cycle since the XPC-deficient BM cells were defective in G1 checkpoints but had an increased accumulation in G2 compared to wild type. Moreover, colony-forming ability of XPC-deficient BM cells was decreased 3-fold compared to wild type even in the absence of carboplatin. The molecular analysis of BM cells of *Xpc* KO mice treated or untreated with carboplatin showed a defective ubiquitinylation of CUL4A and CDT1 cell cycle protein which was associated to role of XPC in signaling of oxidative base damage (Arias and Walter, 2007; Hu et al., 2004; Sugasawa et al., 2005). Finally, the importance of this study lies in favor of cancer chemotherapy where it showed for the first time that the GGR pathway and more specifically XPC is a protective mechanism in BM which is often dose limiting in response to cancer chemotherapy drugs including carboplatin (Fischer et al., 2009).

B. XPC function in leukemia

1. XPC implication in AML predisposition

Acute myeloid leukemia (AML) is the most common acute leukemia in adults with estimated 13290 new cases diagnosed and 8820 deaths in 2008. Five-year survival for adult AML is

estimated to be only 22% even when 50–75% of patients achieve complete remission (CR) following induction. Acute Myeloid Leukemia (AML) is frequently associated with genetic abnormalities (Tallman et al., 2005). Several studies have showed that variations in genes of NER pathway leading to inter-individual differences in DNA repair capacity play an important role in AML outcome altering susceptibility to the genotoxic effects of treatment.

Strom et al. studied the role of 6 polymorphisms (*ERCC1* Gln504Lys, *XPB* Lys751Gln, *XPC* Ala499Val, *XPC* Lys939Gln, *XPG* Asp1104His, and *CCNH* Val270Ala) within NER pathway on overall and disease-free survival among 170 adult *de-novo* AML patients with intermediate cytogenetics, treated with induction chemotherapy. They found that the risk of dying among patients carrying at least one variant *XPB* Lys751Gln allele (AC/CC) was 73% higher than among patients with the *XPB* Lys751Gln wild-type (AA) genotype (HR 1.73, $p = 0.03$). For *XPC* Ala499Val, carrying one or two variant alleles (CT/TT) was associated with 78% increased mortality compared to the wild-type genotype (CC) (HR 1.78, $p = 0.02$). Patients with both variant alleles (TT) had a greater than two-fold increased mortality (HR 2.40, $p = 0.02$). Among diploid patients, after adjusting for clinical and socio-demographic variables, patients carrying both *XPB* AC/CC and *XPC* CT/TT had a greater than two-fold increased risk of dying compared to those with the wild-type genotypes (HR=2.49; 95%CI: 1.06–5.85) (Strom et al., 2010).

2. XPC implication in CML predisposition

Chronic myeloid leukemia (CML) results from the clonal expansion of pluripotent hematopoietic stem cells containing the BCR-ABL fusion gene that promotes their proliferation and viability through activating a number of intracellular signal pathways (Melo and Barnes, 2007). BCR-ABL induces genomic instability (Calabretta and Perrotti, 2004) by increasing levels of ROS causing oxidative DNA damage as well as by interfering with repair of DSBs (Canitrot et al., 2003; Deutsch et al., 2001; Fernandes et al., 2009; Stoklosa et al., 2008) that if unrepaired can contribute to the emergence of resistant clones to the ABL kinase inhibitors such as imatinib (Cramer et al., 2008; Dierov et al., 2009; Koptyra et al., 2006). Imatinib is a remarkably effective therapy for chronic phase CML, permitting long-term disease control in about 75% of patients (Druker et al., 2006). Several studies have reported relation between NER pathway and CML. For instance, BCR-ABL regulates the NER pathway (Friedberg, 2001; de Laat et al., 1999), and more specifically, BCR-ABL was shown to interact with the ERCC3 (XPB) protein, forming a complex that is critical for triggering the

initial steps of NER (Canitrot et al., 2003). Moreover, imatinib, the CML treatment drug, was shown to decrease the efficacy of NER in leukemic cells expressing BCR-ABL (Sliwinski et al., 2008). In this sense, genetic polymorphisms in the NER pathway were found to influence the results to imatinib treatment in CML.

In the study of Guillem et al., a comprehensive analysis of 14 SNPs in genes involved in the NER pathway (*ERCC2-ERCC8*, *RPA1-RPA3*, *LIG1*, *RAD23B*, *XPA*, *XPC*) was performed in 92 early chronic phase CML patients to assess their potential relationship with imatinib treatment results. They found that *ERCC5* and *XPC* SNPs correlated with the response to imatinib. Haplotype analysis of *XPC* showed that the wild-type haplotype (499C-939A) was associated with a better response to imatinib whereas heterozygous carriers of TA/CC haplotype were particularly prone to achieve less favorable responses to imatinib. Moreover, the 5-year failure free survival for CA carriers was significantly better than that of the non-CA carriers (98% vs. 73%; $P = 0.02$). In the multivariate logistic model with genetic data and clinical covariates, the *XPC* haplotype was associated with the treatment response, with patients having a non-CA *XPC* haplotype (OR = 4.1, 95% CI 1.6–10.6) being at higher risk of suboptimal response/treatment failure. A potential explanation for how the *XPC* haplotype variants can influence the response to imatinib treatment is that on one hand overexpression of BCR-ABL increases the rate of DNA DSBs through the generation of ROS and on the other hand *XPC* patients lacking a copy of the CA haplotype are less active in DNA repair than the wild-type leading to diminished NER efficacy, higher genomic instability, accumulation of mutations and chromosomal aberrations in the CML cells, conferring them additional proliferative advantages by BCR-ABL independent mechanisms that could eventually promote imatinib resistance (Guillem et al., 2010).

XII. Our research team findings on XPC and oxidative stress

Our lab has made major contributions in the study of XPC in correlation with hypoxia, response to UV exposure, metabolic alterations and carcinogenesis in human keratinocytes. Rezvani and his colleagues have showed that HIF-1 α regulates the expression of XPC and XPD, two important constituents of NER pathway, in UV-irradiated human keratinocyte. Early UVB exposure, proven previously to accumulate cytoplasmic ROS and downregulate HIF-1 α , allows the binding of Sp1 protein, which competes with HIF-1 α for overlapping binding sites on promoter of *XPC*, allowing increased *XPC* mRNA expression. Late UVB exposure, proven previously to accumulate mitochondrial ROS and to enhance phosphorylation of HIF-1 α protein, allows the direct binding of HIF-1 α to a separate hypoxia response element (HRE) in the *XPC* promoter region allowing increased *XPC* mRNA expression. In addition, the promoter region of XPD has been found to contain seven overlapping HREs allowing its regulation by HIF-1 α . Furthermore, several other DNA repair proteins such as XPB, XPG, CSA and CSB were shown to contain putative HREs suggesting that HIF-1 α is a key regulator of the DNA repair machinery. Analysis of the repair kinetics of 6-4 photoproducts and cyclobutane pyrimidine dimers also revealed that HIF-1 α downregulation led to an increased rate of immediate removal of both photolesions but attenuated their late removal following UVB irradiation, indicating the functional effects of HIF-1 α in the repair of UVB-induced DNA damage (Rezvani et al., 2010).

Furthermore, silencing of *XPC* in normal human keratinocytes appeared to activate NADPH oxidase-1 (NOX-1) which upregulates ROS production. Impaired NOX-1 activation abrogates both alteration in ROS levels and modifications of energy metabolism while overexpression of antioxidant enzymes such as catalase, CuZnSOD, or MnSOD was inefficient to block the metabolism remodeling (Rezvani et al., 2011a).

Moreover, further molecular analysis on XPC silencing effect on activation of NOX1 in human keratinocytes has been performed. The result showed that *Xpc* knockdown accumulated DNA damage which increased DNA-dependent protein kinase activity, which subsequently activated AKT1 and NOX1, resulting in ROS production and accumulation of specific deletions in mitochondrial DNA (mtDNA) over time. Interestingly, the *XPC* silenced keratinocytes appeared to exert a tumorigenic potential since its subcutaneous injection into immunodeficient mice led to squamous cell carcinoma formation which could be reversibly blocked by simultaneous knockdown of either NOX1 or AKT (Rezvani et al., 2011b).

XIII. Objectives of thesis project

The importance of DNA repair mechanisms in protection of HSC against the continuous exposure to stress factors throughout lifetime has been the spotlight of research over several years. Among the various DNA damage repair pathways, NER seems to be a hot target for research being modulated during differentiation of neurons (Noussipiel and Hanawalt, 2000a) and more importantly during differentiation of HSC. A previous study by Rossi and his colleagues reported that the investigation of hematopoiesis in young and aged NER-deficient mice models (*Xpd* KO) showed that the functionality and reconstitution ability of HSC is lost with age but not the number (Rossi et al., 2007b). Further study on hematopoietic cell lines by Hsu and his colleagues in the same year showed that NER is modulated with differentiation (Hsu et al., 2007). Nevertheless, NER pathway encompasses two pathways TCR and GGR. At the beginning of our work, there were few studies assessing the functional role of GGR pathway on maintenance of HSC. Therefore, in our project, we have been interested in decoupling the two mechanisms of NER pathway. We propose to study impact of GGR deficiency on HSC maintenance in mice and human models. We hypothesize that cells accumulate more mutations in absence of XPC which will lead to loss of hematopoietic stem/progenitor cell (HSPC) functions, accelerated aging and increased risk of carcinogenesis. As a hypothetical model, we assume that XPC knockdown / knockout will lead to oxidative stress by elevated production of ROS mainly by NADPH oxidase family spanning the cellular membrane but also by mitochondrial metabolism. XPC deficiency might result in accumulation of DNA damage in the HSC nucleus mainly and in the mitochondria which might also lead to increased ROS production. Vice versa, elevated ROS might trigger further DNA damage. Based on the literature, we propose that ROS will induce p21, p19 and p16 that might lead to senescence or induce p53 that might lead to apoptosis (Blanpain et al., 2011; Rezvani et al., 2011a; Suda et al., 2011). Then, accumulated DNA damage in nucleus might lead to malignant transformation of HSC generating leukemias or in case of survival could lead to aging. Thereby, we propose that XPC deficiency due to inhibition or knockdown disrupts maintenance of HSCs (**Figure 25**).

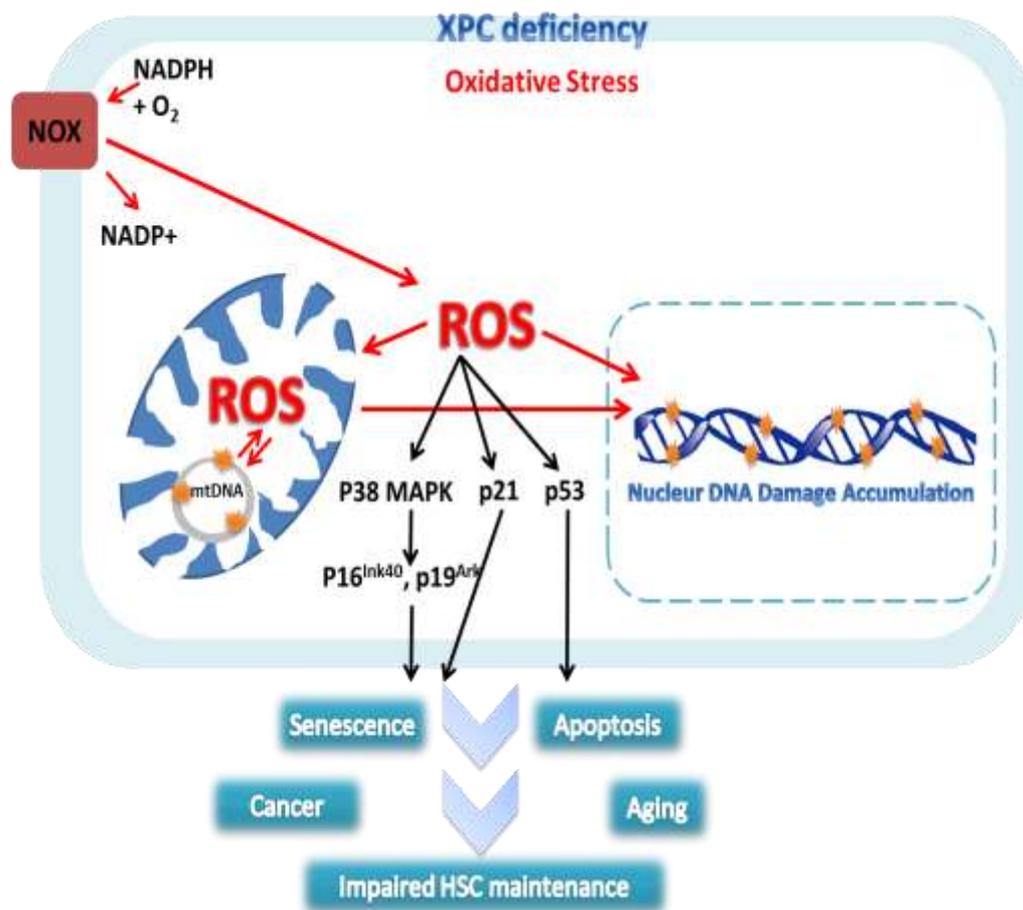


Figure 25. Hypothetical model for XPC effect on HSC.

We have integrated in our project the use of both mouse and human model to study XPC in HSC maintenance and differentiation. Indeed, for the mice study we will investigate the *Xpc* KO mouse model and for the human study we will investigate the UCB-derived CD34⁺ cells silenced for *XPC* by lentiviral transduction.

Our project aims to study the effect of XPC, a major protein in the NER pathway and the cornerstone of GGR, on HSC fate, using *in vitro* and *in vivo* model systems. Therefore, we define our specific aims as follows:

Aim1: To characterize XPC expression profile of different hematopoietic compartments.

Aim2: To determine whether XPC expression is affected by the differentiation of HSC.

Aim3: To study the effects of XPC silencing on HSC properties such as self-renewal, proliferation, reconstitution ability, and clonogenicity.

Aim4: To check whether XPC deficiency might impact hematopoietic cell composition (from stem to mature cells) in mice and if it is related to aging.

Aim5: To investigate whether XPC deficient bone marrow cells are affected in their reconstitution ability.

Aim6: To determine whether *XPC* silencing can itself induce leukemogenesis.

Chapter 2

MATERIALS AND METHODS

The thesis project is divided into two major parts. The first part of the project is a “Human Study” which consists of studying the expression and role of XPC in human hematopoiesis by knocking down (KD) *XPC* gene in UCB-derived CD34⁺ cells. The second part is a “Mouse Study” which consists of characterizing murine hematopoiesis in an aged *Xpc* knockout (KO) model. Both *in vitro* and *in vivo* experiments are performed to address the questions.

I. Human Study

A. Purification of CD34⁺ cells from UCB

1. Collection of UCB samples

UCB samples are obtained from normal full-term deliveries with signed informed consent. The blood samples are collected in PBS supplemented with EDTA (2 mM), Heparin choay (Sanofi Aventis, Paris, France), and Penicillin/Streptomycin (Gibco Life Technologies™, USA). Then, they are stored at 4°C and processed within 24 to 48 h after the delivery.

2. Mononuclear cells (MNC) isolation

UCB samples are first diluted by adding an equal volume of PBS-EDTA buffer. Then, MNC, from individual or pooled samples, are isolated by centrifugation using Ficoll (Eurobio, Les Ulis, France) density gradient. After that, the collected MNC are washed twice with PBS-EDTA buffer.

3. Magnetic enrichment of CD34⁺ cells

First, MNCs are washed with PBS-EDTA containing 0.5% Human Serum Albumin (HSA) to block non-specific adhesion sites of antibodies. CD34 cell enrichment is then achieved by EasySep™ positive selection (Stem Cell Technologies, Vancouver, Canada) according to the manufacturer instructions. In brief, MNCs are first incubated for 15 min at room temperature (RT) with EasySep® Positive Selection Cocktail (anti-CD34) at 100 µL/mL of cells followed by second incubation for 10 min at RT with EasySep® Magnetic Nanoparticles at 50 µL/mL of cells. After this, EasySep® Magnet is used to retain the magnetically labeled CD34⁺ cells which are later washed for 6-7 times. Finally, isolated CD34⁺ cells are counted and labeled with mouse anti-human CD34 antibody conjugated to phycoerythrin (cat# 12-0349-42, CD34-PE, eBioscience) to check for the purity. The isotype control used is mouse immunoglobulin-

G (IgG1 k) conjugated to PE (cat# 12-4714-82, Iso-PE, eBioscience). 1 $\mu\text{g}/\text{mL}$ 4,6-diamidino-2-phenylindole (DAPI) is used to exclude the non-viable cells. Flow analysis is performed on FACS CantoII™ (Becton Dickinson, San Jose, CA, USA) using Diva software.

CD34⁺ samples that reach 92 to 99% purity are stored in liquid nitrogen for further use or are pelleted for molecular analysis (**Figure 26**).

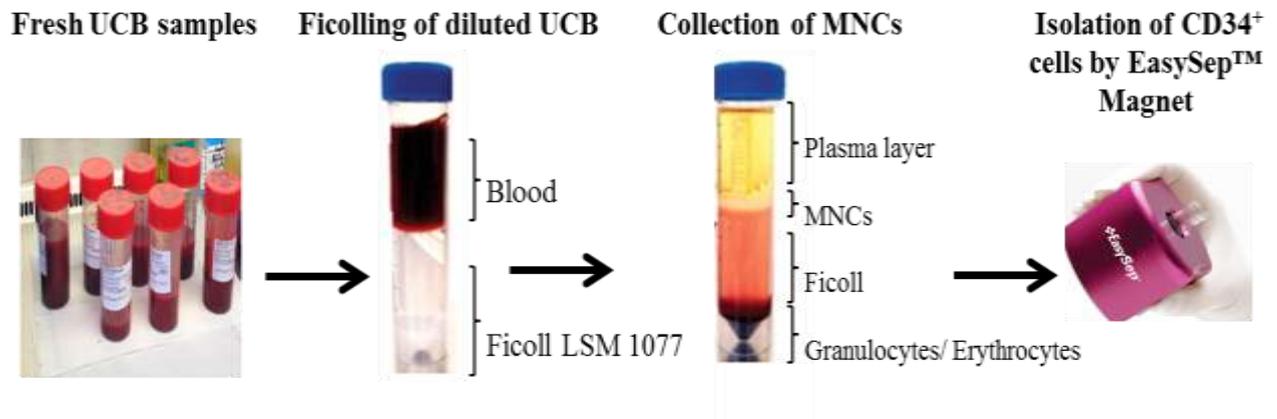


Figure 26. Immuno-magnetic isolation of CD34⁺ cells from human UCB. Schematic representation for the purification of CD34⁺ cells from human UCB by ficolling and EasySep™ magnet.

B. Generation of lentiviral vectors and particles

1. Production of Plasmids

Vectors expressing shXPC, shLuc and shRFP were previously synthesized in the laboratory by inserting enhanced green fluorescence protein (GFP) cDNAs into the multiple cloning sites (MCS) of a TPW vector downstream of the human phosphor-glycerate kinase (hPGK) promoter. TPW plasmid was then used as the backbone for the construction of the shXPC by replacement of the hPGK promoter with the EF-1 α promoter and insertion of a cassette containing the H1 promoter followed by shRNA sequence targeting the XPC protein (Rezvani et al., 2011a). An shRNA directed against the dsRed fluorescent (RFP) protein (Rezvani et al., 2011a) or against the luciferase (Luc) protein (Lafitte et al., 2012) was used as control (shCtrl). The map of the plasmids is listed below (**Figure 27**).

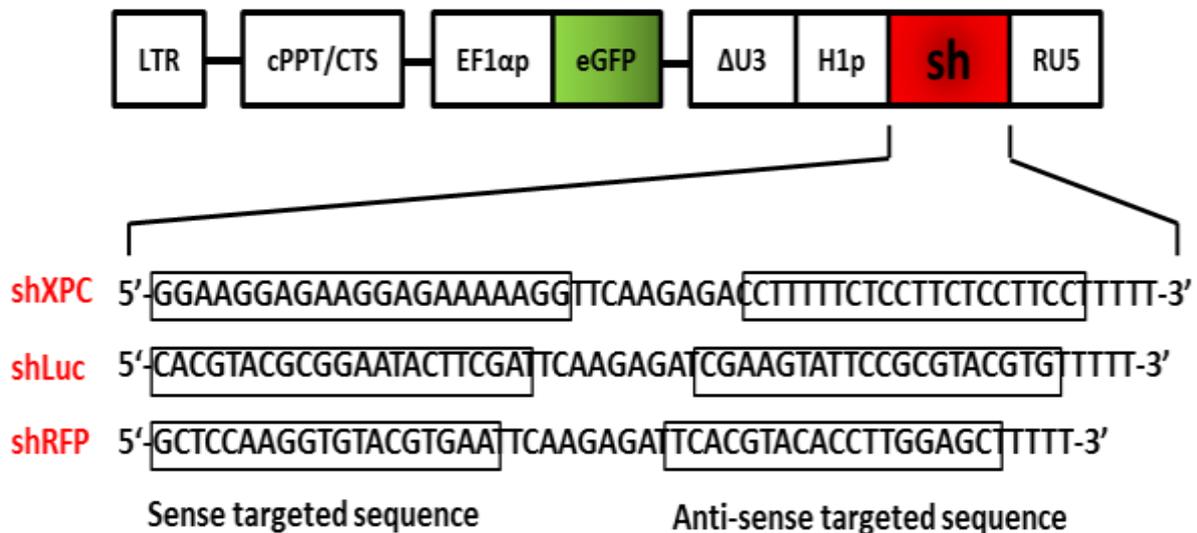


Figure 27. shXPC and shCtrl vector constructs. Schematic representation of lentiviral construct used to inhibit XPC, Luc and RFP (control) expression is shown. The vector carries an internal cassette for the enhanced green fluorescent protein (GFP) driven by the EF-1 α promoter and a cassette containing the H1 promoter followed by a shRNA sequence targeting the XPC or Luc/RFP mRNA. Δ U3, R, and U5 are the LTR regions, with a deletion that includes the enhancer and the promoter from U3. cPPT is a nuclear import sequence.

2. Amplification of Plasmids

STBL2 electrocompetent bacteria are produced by Max Efficiency STBL2 kit (cat# 10268, Invitrogen) according to the manufacturer's instructions. Transformation of STBL2 bacteria with the plasmids is done by electroporation technique in flame-sterile conditions. Briefly, the bacteria are transfected by adding 1 μ L of shLuc, shXPC, VSVG (vesicular stomatitis virus glycoprotein envelope expressing plasmid pMD.G) or PAX (packaging construct pCMVPAX2) plasmids to 50 μ L of these bacteria. Then, the plasmids and the bacteria are added into 2 mm electroporation cuvettes (cat#5520, Molecular Bioproducts), electroporated, and collected by adding SOC media. After that, bacteria are spread on an agar plate containing ampicillin (50 μ g/mL) and the plates are incubated overnight at 37°C. After 24 h, one colony is dispensed in 400 mL Lysogeny Broth (LB) medium supplemented with Ampicillin (50 μ g/mL) and is incubated overnight at 37°C with continuous shaking. A Maxiprep (cat#740424, Nucleo Bond® Xtra Maxi EF, Macherey-Nagel) is then performed according to the manufacturer's instructions. Finally, the DNA pellet is quantified by Nanodrop®.

3. Plasmid Quality Verification by restriction enzyme digestion

The plasmid quality is verified by restriction enzyme digestion. The shXPC plasmid (4 μ g) is fragmented by XhoI (3 bands are expected) whereas EcoRI is used to fragment 0.5 μ g of

VSVG (1671-4153 bp bands), PAX (3474-6328 bp bands) and shLuc (1174 bp band). Incubation at RT for 1 h is followed. Then the restricted fragments are run on 1% agarose gel for VSVG, PAX and shLuc, and on 2% agarose gel for shXPC. 1 Kb ladder was used to detect size of fragments after gel electrophoresis.

4. Viral Production

Lentiviral particles are produced by transient transfection of HEK (human embryonic kidney) 293T cells using a calcium chloride transfection technique. First, HEK 293T adhesion cell lines are cultured in DMEM medium with 10% fetal bovine serum (FBS) and penicillin/streptomycin (P/S; 10 U/mL). Second, one million 293T cells are plated on 0.01% L-polylysine (cat# P-4832, Sigma) coated 10-cm plates and incubated at 37°C for 3 days. When cell confluency reached 50-70% on the third day, the medium is changed (DMEM, 10% FBS, PS). Prior to transfection, 2.5 M calcium chloride solution and 2x-HBS HEPES buffered saline buffer of exact pH = 7.12 are prepared. The latter consists of 280 mM sodium chloride, 1.5 mM sodium phosphate, and 100 mM HEPES. Twenty four hours later, 293T cells are washed and transfected by calcium chloride technique, using multiple constructs for each petridish: the packaging construct pCMVPAX2 (10 µg), a vesicular stomatitis virus glycoprotein (VSVG) envelope construct pMD.G (4 µg) and a vector construct (shXPC or shLuc/shRFP, 10 µg). After 8 h of transfection, the medium is removed and replaced by serum-free Optimem (Gibco, CergyPontoise, France) supplemented with 2 mM HEPES (Fluka, Lyon, France) and 10 U/mL P/S (Lonza, Basel, Switzerland). Forty eight hours later, viral supernatants are collected into 50 mL falcon tubes, centrifuged to remove the cell debris, filtered through 0.22 µm filters, and concentrated by ultracentrifugation at 35000 x g for 4 h at 4°C. The supernatant is discarded and the lentiviral particles are then resuspended in 200 to 400 µL of StemSpan medium. Produced lentiviral particles are distributed in eppendorf tubes and preserved at -80 °C for long term storage.

5. Virus titer

Infectious titers are determined by transducing 293T cells with serial dilutions of viral supernatant (1/10000 to 1/100). For transduction, 5×10^4 293T cells per well in a 24 well plate are incubated for 15-16 h with the appropriate volume of lentiviral particles in a total volume of 200 µL of DMEM supplemented with 10% FBS-PS. The supernatant is then removed and replaced by 500 µL of the same medium. After three days, the titer is determined

by checking the percentage of GFP⁺ cells by flow cytometry. Finally, virus titer (IP/ml) is calculated as equal to % transduction x initial number of cells x dilution factor x 1000.

C. Liquid culture of CD34⁺ cells

1. CD34⁺ cell culture

The CD34⁺ cells are thawed in FBS (cat# CVFSVF00-01, Eurobio) medium, washed (2 times) and cultured in STEM SPANTM serum-free medium (cat# 09650, SFEM, StemCell Technologies, Canada), supplemented with 50 ng/mL Stem Cell Factor (cat# 300-07, SCF, Peprotech, USA), 50 ng/mL Fms-related tyrosine kinase 3 ligand (cat# 300-19, Flt3-L, Peprotech, USA), 50 ng/mL Thrombopoietin (cat# 300-18, TPO, Peprotech, USA), 1% P/S (10 U/ml) and 1 μM of aryl hydrocarbon receptor antagonist Stem Regenin 1 (cat#1967-1, SR1, Biovision Incorporated, Milpitas, CA95035, USA). Cells are cultured at 37°C in humidified atmosphere with 20% O₂ and 5% CO₂ for 14 days. At different time points, day 4-7-11-14, cells are collected, pelleted and stored at -80°C for further molecular analysis.

2. CD34⁺ cell transduction

CD34⁺ cells are transduced with shXPC and shCtrl vectors using a M.O.I. = 100 and cultured in complete BIT medium (cat#09500, Bovine serum albumin/Insulin/Transferrin, BIT 9500 Serum Substitute, Stem CellTM Technologies) supplemented with necessary cytokines at concentration of 10⁶ cells/mL for 15-16 h at 37°C in a fully humidified atmosphere (20% O₂ and 5% CO₂). Later, the transduced cells are washed twice with IMDM and resuspended in complete Stem Span medium at concentration 10⁵ cell/mL. After 4 days of transduction, the percentage of GFP-expressing cells is determined by flow cytometry.

3. GFP stability assay

Transduced CD34⁺ cells by shXPC or shCtrl vectors are seeded in culture for 2 weeks in STEMSPAN supplemented with cytokines and SR1. The amplification of cells is assessed by trypan blue exclusion method and the GFP stability is assessed by flow cytometry that allowed the detection of GFP-expressing cells.

D. Progenitor cell assay

Committed progenitors are analyzed by seeding 500 transduced CD34⁺ cells in semi-solid culture medium with recombinant cytokines known as StemAlpha 1D (cat# 5112, Stem Alpha SA, La Chenevatiere, France). Cells are seeded after 1 or 4 days of culture of transduced CD34⁺ cells with shXPC or shLuc lentiviruses. Burst forming unit-erythroid (BFU-E) and colony forming unit-granulocyte macrophage (CFU-G, CFU-M, CFUGM) derived colonies are scored according to their morphology at 14 days following plating using an inverted microscope (**Figure 28**).

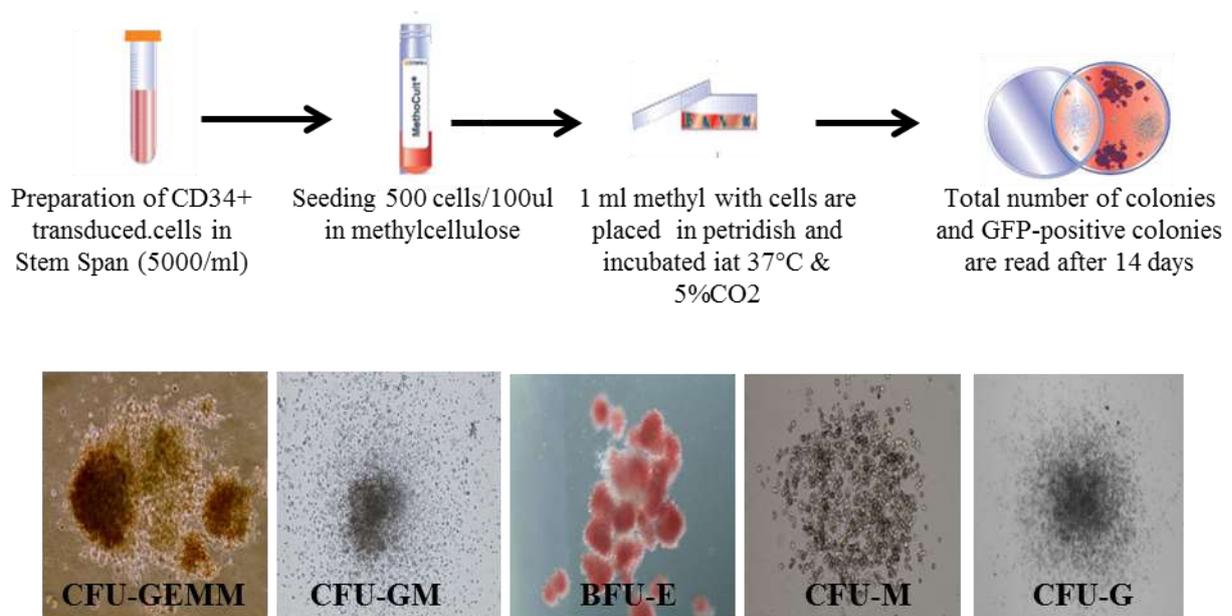


Figure 28. Progenitor cell assay of transduced CD34⁺ cells. Procedure of methylcellulose assay (top image) and inverted microscopic images of methylcellulose observed colonies produced by CD34⁺ cells (bottom image). *Symbols: BFU-E: Burst forming unit-erythroid; CFU-G/M: colony forming unit-granulocyte/macrophage.*

E. Flow cytometry

Flow cytometry analysis is performed using a FACScalibur™ (Becton Dickinson, San Jose, CA, USA). Cell sorting is performed using a FACS Aria (Becton Dickinson) that was used to sort GFP-expressing cells after 4 days of CD34⁺ transduction with shLuc and shXPC vectors and to sort CD34-expressing cells and CD34-deficient cells (CD34⁺/CD34⁻) after 11 days of liquid culture of CD34⁺ cells.

F. XPC protein expression

1. Protein isolation

Prior or after culture, cells are pelleted (1 million cells/sample). The cell lysis buffer (RIPA) is composed as follows: 50 mM Tris-Cl pH7.5, 150 mM NaCl, 1% triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and 0.1 mM PMSF and cocktail of protease inhibitors. Forty microliters of lysis buffer is added to each cell pellet followed by short vortexing and 30 min of ice incubation. After centrifugation at 12000 x g for 10 min, the supernatant containing the proteins is collected and stored at 4°C before processing.

2. Protein Dosage assay

Protein dosage is performed using ‘Pierce® BCA Protein Assay’ kit (cat#23227, ThermoScientific, Rockford, USA) according to the manufacturer’s instructions. In brief, serial dilutions of the standard BSA albumin protein (125-2000 µg/mL) are prepared in order to plot the standard curve. Ten microliters of each BSA concentration are distributed in a 96-well plate. Each lysate is diluted in lysis buffer to a final volume of 10 µL/well. Next, 200 µL of the working reagent (Solution A+1/50 Solution B) are added into each well followed by 30 min incubation at 60°C. Noteworthy BCA reagent A contains sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1M sodium hydroxide while BCA Reagent B contains 4% cupric sulfate. The optical density is measured by spectrophotometer using a wavelength $\lambda=550$ nm.

3. Western blotting

a. Gel preparation

First, the migration 8% SDS polyacrylamide gel is prepared consisting of 30% acrylamide, 1.5 M Tris pH 8.8, 10% SDS, 10% ammonium persulfate and TEMED. Second the stacking gel is prepared consisting of 30% acrylamide solution, 1 M Tris pH 6.8, 10% SDS, 10% ammonium persulfate and TEMED.

b. Protein loading and migration

Protein samples are prepared in Laemmli loading buffer before separation by SDS-PAGE electrophoresis that allows separation of proteins according to their molecular weight. The laemmli loading buffer constitutes of 60 mM Tris-Cl pH 6.8, 2% SDS, 10% glycerol, 5% β -mercaptoethanol and 0.01% bromophenol blue. Total protein extracts (25 µg) are loaded into

the wells of stacking gel. Then proteins are migrated on 8% SDS polyacrylamide gel. Migration is performed at 25 mA until bromophenol blue reaches the bottom of the gel.

c. Protein Transfer

Proteins are transferred by wet transfer to nitrocellulose membranes at 100 volts during 90 min. The efficiency of transfer is confirmed by staining the membrane with Ponceau red. Then, the membrane is treated with blocking solution: 5% fat-free milk prepared in 0.1% Tween20-TBS for 1 h at RT. The blocking solution contains casein proteins that bind sites on the membrane that could be non-specifically recognized by antibodies. The membrane is incubated overnight at 4°C with monoclonal antibodies (MAb) directed against the C-terminus of the human XPC (clone 3.26, cat# GTX 70294, GeneTex) or alternately the rabbit polyclonal anti-XPC (cat# GTX 70308, GeneTex) antibody directed against the N-terminus, all diluted at 1:1000. β -Actin is used as control for equal loading by the use of mouse anti-Actin MAb used at 1:10,000 dilution. XPC and Actin are revealed after secondary incubation for 2 h with mouse or rabbit anti-immunoglobulin horseradish peroxidase (HRP)-linked antibody used at dilution of 1:5000 and 1:10,000, respectively. The molecular weight marker (Euromedex) enabled the determination of protein sizes. Blots are developed using the chemiluminescence ECL reagent Kit (Bio-Rad). Quantification of XPC and actin bands are carried out by densitometry (Fujifilm™ LAS-3000 Imager).

G. XPC RNA expression

1. RNA isolation

Total cellular RNA is extracted using Trizol (Invitrogen, CA) according to the manufacturer's instructions. In brief, Trizol reagent is added (1 mL) over the sample to disrupt cells and dissolve cell components. Then, chloroform is added followed by centrifugation (12000 X g, 15 min). Aqueous phase is collected and RNA is recovered by precipitation with isopropanol. The precipitated RNA is washed with 70% ethanol and finally dissolved in RNase-free water previously supplemented with 0.1% Diethylpyrocarbonate (DEPC) (cat#D-5758, Sigma, St.Louis, USA). The quantity of RNA is assessed for by Nanodrop. Pure RNA is achieved when $OD_{260/280}$ is superior to 1.8.

2. RNA quality control

Integrity of RNA is assessed denaturing electrophoresis. First, 1 µg RNA is loaded on ethidium bromide-stained 1% agarose gel. All materials are previously washed (> 2 hours) with 0.4M NaOH to inhibit RNases. The gel is left to migrate at 100 V for 30 min. Purity is estimated by the presence of two bands representing 28S rRNA (5034 bases) and 18S rRNA (1870 bases).

3. RT-PCR

Total cellular RNA (500 ng) is reverse transcribed using the Transcription First Strand cDNA synthesis kit (Roche Version 6, Germany) according to the manufacturer's instructions. RNA is incubated with oligodT or random hexamer primer for 10 min at 65°C then is incubated on ice for 5 min. After that, mixture of 5x Reaction buffer, deoxynucleotides, Rnase inhibitor and Transcriptor Reverse Transcriptase is added. The RT-PCR reaction constituted of 3 steps: 10 min at 25°C, 1 h at 50°C and 5 min at 85°C.

4. qRT-PCR

Quantitative Real-time PCR (qRT-PCR) is carried out for *XPC* and *tubulin* using Sybr Green QPCR Master Mix (Agilent Technologies, USA) with an ABI Thermal cycler. The reactions are cycled 40 times after initial polymerase activation (95°C, 3 min) using the following parameters: denaturation at 95°C for 15 s, annealing at 60°C for 22 s. A final fusion cycle (95°C, 1 min; 60°C, 30 s; 95°C, 30 s) terminated these reactions. The sequences of the *XPC* and *tubulin* primers are listed below (**Table 5**). The standard curve is made by using pooled CD34+ sample demonstrating a linear relationship between the Ct values and the cDNA concentration. The relative expression of *XPC* gene is assessed by considering the Ct and efficiency values and normalized according to the tubulin expression level.

Target gene	SEQUENCE
XPC	F: GGTGAGGCTTGGAGAAGTAC
	R: ACATTCCCAAACCTCGTTCCG
Tubulin	F: GAGTGCATCTCCATCCACGTT
	R: TAGAGCTCCCAGCAGGCATT

Table 5. XPC and Tubulin primer sequences. Symbols: F: Forward primer; R: Reverse primer.

H. NOD-scid IL2Rg^{null} mice repopulation assay

1. Long term repopulation (LTR) assay

In agreement with Bordeaux University's ethical committee board, transduced CD34⁺ cells are transplanted into 7- to 10-week old NOD-scid IL2Rg^{null} (NSG) mice (Jackson Laboratory, Bar Harbor, Maine, USA) using intravenous injection for primary and secondary mice as previously described (Robert-Richard et al., 2010). Prior to transplantation, mice received a preparative conditioning regimen consisting of a 3-day intraperitoneal injection of 22 mg/kg busulfan (Busilvex, Pierre Fabre, Boulogne, France) as previously described (Robert-Richard et al., 2006). BM samples are aspirated from the right femur (RF), under isoflurane anesthesia, 3, 6 and 12 weeks after transplantation. Mice also received Vetegesic® (Cetravet SA Cooperative, Dinan, France) at 100 µg/kg as an analgesic. 15- to 24- weeks after transplantation, primary mice are sacrificed and human cell engraftment is assessed by flow cytometry on part of the pooled BM cells from femurs, tibia, femur and pelvis. The rest of cells are intravenously injected into busulfan-preconditioned secondary recipients (**Figure 29**).

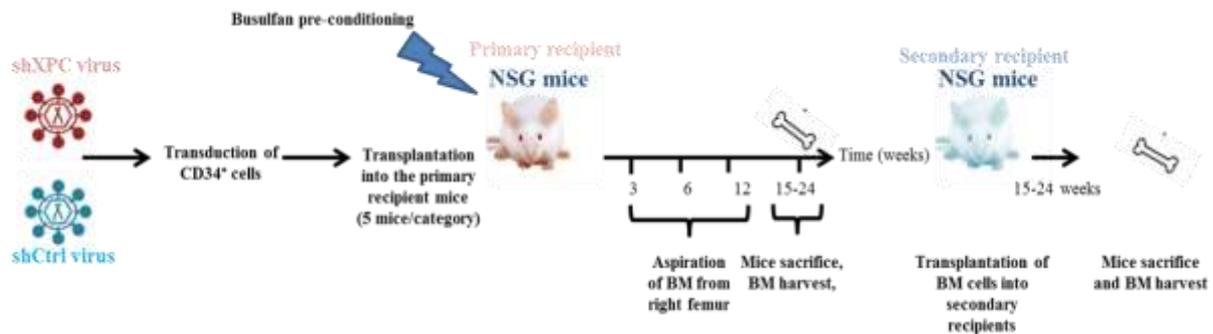


Figure 29. NSG xenotransplantation by shXPC- or shCtrl-transduced CD34⁺ cells and follow-up of human chimerism and GFP stability. Schematic representation showing detailed description for the transplantation experiment conducted on NSG mice. 5 NOD/SCID mice were injected with shXPC-transduced CD34⁺ cells, 5 were injected with shLuc-transduced CD34⁺ cells serving as control as 5 other mice were injected with non-transduced CD34⁺ cells. The kinetics of human GFP⁺ cells was followed with time. Primary and secondary transplantations were performed.

2. Labeling of BM cells

Hematopoietic composition at different time points of transplantation is analyzed by different panels of antibodies directed against cell surface markers that characterize the different subsets of the hematopoietic system. Human CD45-PC7 (cat# 25-0459-42, eBioscience), mouse CD45-eFluor450 (cat# 48-0451-82, eBioscience) and Ter119-eFluor450 (cat# 48-5921-82, eBioscience) antibodies allowed distinguishing human cells from the mouse cells.

Erythroid lineage is studied using CD36-PE (cat# 555455, BD Pharmingen) and GPA-APC (cat# 17-9987-42, eBioscience) antibodies. Myeloid lineage is studied using CD33-APC (cat# 551378, BD Pharmingen) and CD14-APCVio770 (cat# 130-096-622, MACS) antibodies. Lymphoid lineage is studied by the use of CD3-APC (cat# 17-0038-42, eBioscience), CD19-PE (cat# 555413, BD Pharmingen) antibodies. Stem cells are studied by primary incubation with human lineage/biotin antibody cocktail (cat# 120-001-875, MACS) used to mark mature cells, followed by secondary incubation with antibiotin-Vioblue (cat# 130-094-669, MACS), CD34-PE (cat# 12-0349-42, eBioscience), CD38-APC (cat# 555462, BD Pharmingen), and CD45RA-Viogreen (cat#130-096-921, MACS) antibodies. CD16/32 antibody is used within all our panels to block non-specific binding of antibodies. In addition, panel of isotype antibodies is used to set up the gate threshold: IgG2bk Iso-eFluor450 (cat# 48-4031-82, eBioscience), Mouse IgG1K Iso-APC (cat# 17-4714-82, eBioscience), Iso-PC7 (cat# 25-4714-42, eBioscience) and Iso-PE (cat# 12-4714-82, eBioscience). One hundred thousand cells were used for FACS acquisition.

3. ROS measurement on Lin⁻CD34⁺GFP⁺ BM cells from NSG mice

One million BM cells from each NSG mice are used to measure ROS production in human transduced stem cells Lin⁻CD34⁺GFP⁺ after 18 weeks of transplantation. First BM cells are incubated with 2.5 μ M CellROX probe (cat#C10422, Life Technologies) for 20 min at 37°C. Biotinylated anti-lineage antibody is added to the cells to label lineage-positive cells for 20 min incubation at 4°C. Cells are washed with PBS and then incubated with antibiotin-vioblue and CD34-PE antibodies for 20 min at 4°C. After that, Cells were washed and analyzed by flow cytometry.

I. Engraftment analysis of organs

1. Spleen processing

Spleen of each mouse is collected in 2 ml IMDM medium. Then the spleen is smashed by the use of 40 μ M nylon cell strainer “Tamis” and 5 ml syringe to extract its cells which are collected in IMDM medium. The cells are counted and 150,000 cells are used for the analysis.

2. Thymus processing

Thymus of each mouse is collected in 2 mL IMDM medium. Thymus is smashed in 200 μ L IMDM by needle and syringe. Directly, without numeration, 50 μ L of cells are used for the analysis.

3. PB processing

PB is collected by heart puncture under anesthesia in 3 mL PBS/EDTA. Then it is lysed by 2 rounds of incubation with 10 mL lysis buffer for 10 min during which the tubes are mixed several times. After centrifuge, pellet is resuspended in 200 μ L PBS/EDTA and 60 μ L of cells are used for the analysis.

J. Liquid culture of cell lines

1. Collection of leukemic cell lines

15 Acute Myeloid Leukemia (AML) cell lines classified in seven different subtypes (M0, M2, M3, M4, M5 and M7) were used in our study. The subtypes of AML, M0 through M7, were created by the French-American-British (FAB) classification system based on the type of cells from which the leukemia developed and how mature the cells are. From the M0 subtype (Acute Myeloblastic Leukemia with minimal differentiation), KG-1 and KG-1a (a subclone of KG-1) Erythroleukemic cell lines of BM origin were used. From the M2 subtype (Acute Myeloblastic Leukemia with maturation), HL-60 Acute Promyelocytic Leukemia cell line (APL) and KASUMI-1 AML cell line, both of PB origin were used. From M3 subtype (Acute Promyelocytic Leukemia), NB-4 APL cell line of BM origin was used. From M4 subtype (Acute Myelomonocytic Leukemia), ML-2 and OCI-AML3 AML cell lines originating both from PB were used. From M5 subtype (Acute Monocytic Leukemia), MOLM13 AML cell line, MV4-11 and THP-1 Acute Monocytic Leukemia cell lines all of PB origins were used in addition to U-937 Myelomonocytic Histiocytic Lymphoma cell line originating from pleural effusion. From M6 subtype (Erythroleukemia), HEL and TF-1 Erythroleukemia cell lines originating from PB and BM respectively were used. From M7 subtype (Acute Megakaryocytic Leukemia), M-O7e Megakaryoblastic Leukemia cell line and UT-7 AML cell line were used originating from PB and BM respectively. Moreover, other than AML cell lines, K562 Chronic Myeloid Leukemia (CML) cell line originating from pleural effusion and NALM-6 Acute Lymphoblastic Leukemia (ALL) cell line originating from PB were also used

in the study (Table 6). The information on cell lines was collected from <http://www.proteinatlas.org>.

Cell line	AML FAB Subtype	Description	Origin
KG-1	M0	Erythroleukemia cell line	Bone Marrow
KG-1a	M0	Erythroleukemia cell line; subclone of KG-1	Bone Marrow
HL-60	M2	Acute promyelocytic leukemia (APL) cell line	Peripheral blood
KASUMI-1	M2	Acute myeloid leukemia (AML) cell line	Peripheral blood
NB-4	M3	Acute promyelocytic leukemia (APL) cell line	Bone Marrow
ML-2	M4	Acute myeloid leukemia (AML) cell line	Peripheral blood
OCI-AML3	M4	Acute myeloid leukemia (AML) cell line	Peripheral blood
MOLM-13	M5	Acute myeloid leukemia (AML) cell line	Peripheral blood
MV4-11	M5	Acute monocytic leukemia cell line	Peripheral blood
THP-1	M5	Acute monocytic leukemia cell line	Peripheral blood
U-937	M5	Myelomonocytic histiocytic lymphoma cell line	Pleural effusion
HEL	M6	Erythroleukemia cell line	Peripheral blood
TF-1	M6	Erythroleukemia cell line	Bone Marrow
M-07e	M7	Megakaryoblastic leukemia cell line	Peripheral blood
UT-7	M7	acute myeloid leukemia cell line	Bone Marrow
K-562	-	Chronic myeloid leukemia (CML) cell line	Pleural effusion
NALM-6	-	Acute lymphoblastic leukemia (ALL) cell line	Peripheral blood

Table 6. Properties of the used cell lines.

2. Culture of cell lines

Cells are cultured at 37°C in humidified atmosphere with 20% O₂ and 5% CO₂. ML2, HL60, THP1, HEL, MOLM-13, NALM-6, KG1, K562 NB4 and U937 cell lines were cultured in RPMI media supplemented with 10% FBS. MV4-11 and TF1 cell lines are cultured in same manner but medium was also supplemented with 5 ng/mL and 10 ng/mL of GM-CSF respectively. Kasumi-1 cell line is cultured in RPMI medium containing 20% FBS. UT7, KG1a and OCI-AML3 cell lines were cultured in IMDM medium supplemented with 20% FBS. Finally MO7e cell line is cultured in IMDM medium supplemented with 10% FBS, 10 ng/mL GM-CSF and 0.2% β-mercaptoethanol.

3. Transduction of leukemic cell lines

HL60, THP1, U937 and K562 cell lines are seeded at concentration of 10⁵ cells/100 μL. Then cells are transduced with shXPC and shCtrl vectors using an M.O.I. = 4 which gives more than 90% transduced cells.

K. Detection of alternative splicing variants

HL60 cell line is used for the experiment and keratinocyte cells are used as control. This technique involves four major steps. The first is RNA isolation of cells using Trizol reagent. The second step is cDNA synthesis by RT-PCR using 1 μg of RNA. The third step requires

the amplification of the cDNA by carrying out for PCR reactions using 4 consecutive primer couples which allow us to amplify regions A, B, C, D of human *XPC* gene. Thus the whole *XPC* coding sequence will be amplified and that will produce 4 PCR products of different molecular weights where region A is 643bp, region B is 746 bp, region C is 694 bp and region D is 917bp. The primers sequences to amplify region A, B, C and D are listed below (**Table 7**). The PCR reactions were cycled 37 times after initial denaturation (95°C, 15 min) using the following parameters: denaturation at 95 °C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. A final extension cycle (72°C, 4 min) terminated these reactions. Finally, amplified fragments are visualized by ethidium bromide and UV light on 1% agarose gel using 1Kb marker.

Target gene	SEQUENCE
XPC region A	F: GTGGAATTTGCCAGACAAG R: TTGTGTGTGTCCTCATGGAC
XPC region B	F: CTGGAGTTTGAGACATATC R: CTCTTTATAAGACACCCTGG
XPC region C	F: CAAGTGCCAAAGGGAAGAG R: GTATGGTCTCAAGGTCTCG
XPC region D	F: GAGATGTCACACAGAGGTAC R: CCCTTCAGCTTCTGCTTTTC

Table 7. Sequences of the 4 primer couples used for amplification of human *XPC* cDNA.

II. Mouse Study

A. Breeding and genotyping of *Xpc* KO mice

Xpc KO mice were purchased from University of Texas. Mice were bred and maintained at the animal facility in University of Bordeaux. All mice were on a C57BL/6 background. Animal procedures were carried out in approval with the Bordeaux University ethical committee board.

Noteworthy, at the molecular level, the *Xpc* KO mice are generated by replacement of exons 10 and 11 of the *Xpc* gene by the Neo gene. This knockout system allowed us to identify the different genotypes of the bred mice and was first described by Cheo et al. (Cheo et al., 1997).

In order to genotype the mice, DNA extraction is first performed by overnight incubation of tail samples, from 3-week old mice, in DirectPCR Lysis Reagent (Viagen Biotech, Inc., Los Angeles, CA90010) containing freshly prepared 10 mg/ml Proteinase K (Macherey Nagel, Neamann, Neander, Duren) at 56°C in hybridization oven (Appligene). Lysates are then incubated for 15 min at 95°C to allow denaturation and directly used for PCR. The first PCR aiming to detect the wild-type (WT) gene uses *Xpc* EX and IN primers that generate a 205 bp product. The second PCR aiming to detect the homozygote (Ho) gene uses alternative forward primer *Xpc* NEO primer and the *Xpc* IN primer providing a 390 bp product (sequences are listed in table below). The PCR reactions are cycled 35 times after initial denaturation (95°C, 15 min) using the following parameters: denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 sec. A final extension cycle (72°C, 5 min) terminated these reactions. Extracted PCR-amplified DNA is visualized using 2% agarose gel and 100 bp marker. The primer sequences are listed below (**Table 8**).

Target gene	SEQUENCE
XPC EX	ATTGCGTGCATACCTTGAC
XPC IN	TATCTCCTCAAACCCTGCTC
XPC NEO	CGCATAGCCTTCTATCGCCT

Table 8. Sequence of the XPC EX, IN, and Neo primers.

For our experiments, we selected young WT (yWT) mice of age 9.9±1.6 wks and aged WT (aWT) mice of age 63.8±10.5 wks. For the homozygote mice, the young mice (yHo) are of age 10.3±1.9 wks and the aged mice (aHo) are of age 62.3±9.9 wks (**Figure 30**).

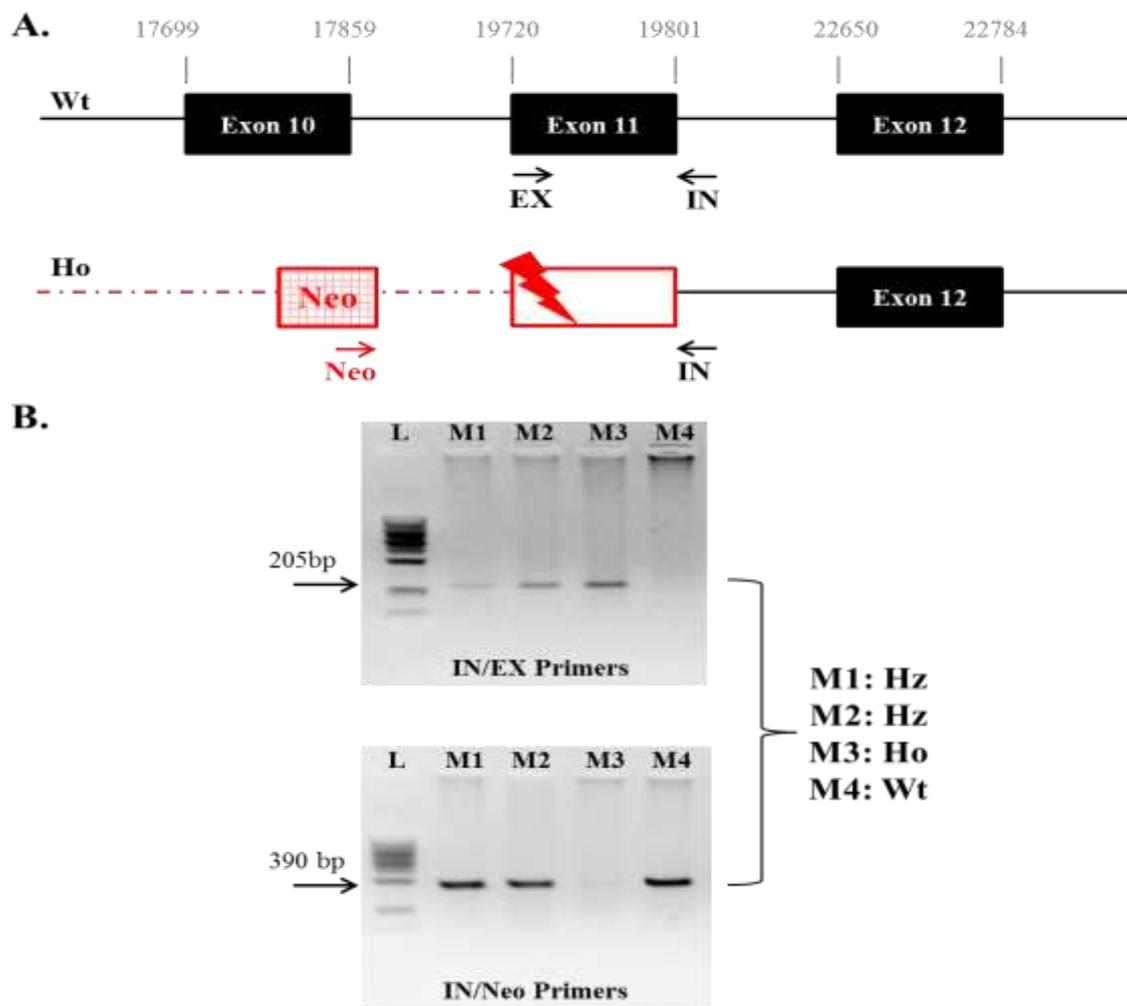


Figure 30. Xpc mice breeding and genotyping. (A) Schematic representation for knockout of exons 10 and 11 in *Xpc* gene in order to produce *Xpc*-knockout mice. (B) Agarose gel image for XPC mice genotyped by PCR using two sets of primers IN/EX and IN/Neo. Symbols: IN: Intron; EX: Exon; Neo: Neomycin; M: mouse; Hz: Heterozygote; Ho: Homozygote; WT: Wild type

B. Weight measurement

Weight of each mouse is measured before sacrifice. The spleen is also collected and its weight is measured.

C. Analysis of mice PB

Peripheral blood from mice is collected from the retro-orbital sinus under anesthesia using heparin capillaries in eppendorf tubes containing 5 μ L of 0.1 mM EDTA.

Next, 75 μ L of blood are lysed in 1 ml of cold lysis buffer containing 0.1 M KHCO₃, 2 M NH₄Cl and 500 mM EDTA. After 10 min of incubation, the samples are washed, centrifuged and resuspended in 150 μ L of PBS/EDTA/HSA.

After that, fifteen μL of cells are used for flow cytometry analysis. Granulocyte cells are detected using anti-Gr1 PE antibody (cat#12-5931-82, eBioscience). Myeloid cells are detected using anti-B220 PE antibody (cat#12-5931-82, eBioscience). Lymphoid cells are labeled by anti-CD3 PE antibody (cat#28005B, BD Pharmingen). Macrophages are detected by labeling with anti-CD11b PE antibody (cat#12-0112-82, eBioscience). Rat IgG2ak Iso-PE antibody (cat# 12-4321-82, eBioscience) is used as isotype control. DAPI was used at 1 $\mu\text{g}/\text{mL}$ in all our tubes to exclude non-viable cells from the analysis. Antimouse CD16/32 antibody (cat# 14-0161-82, eBioscience) is also used to block non-specific binding of antibodies. Analysis of cells is performed by flow cytometry.

D. Analysis of mice BM

Bone marrow from femurs, tibiae and pelvis are flushed and pooled in serum-free medium. Then cells are enumerated in PBS 2% acetic acid containing methylene green (cat# M5015, Sigma). One million BM cells are stained with biotin-conjugated anti-mouse lineage antibodies (Lineage Cell depletion kit mouse, cat#130-090-858, Miltenyibiotec MACS). Then, to identify Common Myeloid Progenitor cells (CMP), lineage-negative cells were stained with antibiotin-vioblue (cat#130-094-669, Miltenyibiotec MACS), CD34-FITC (cat#11-0341-82, eBioscience), CD16/32-PE (cat#12-161-82, eBioscience), Sca1-PC7 (cat#25-5981-82, eBioscience) and ckit-APC (cat#17-1171-82, eBioscience) antibodies. To identify Common Lymphoid Progenitor cells (CLP), lineage-negative cells were stained with Antibiotin-Vioblue, CD135/flk2-PE (cat#12-1351-82, eBioscience), CD127-PerCP-Cy5.5 (cat#45-1271-82, eBioscience), Sca1-PC7, ckit-APC and CD16/32 purified antibodies. To identify Stem cells using LSK method, lineage-negative cells were stained with Antibiotin-Vioblue, CD34-FITC, flk2-PE, Sca1-PC7 and ckit-APC and CD16/32 purified antibodies. In addition, Stem cells are also identified using SLAM method, lineage-negative cells were stained with Antibiotin-Vioblue, CD150-FITC (cat#11-1501-82, eBioscience), CD48-PE (cat#12-0481-82 eBioscience), CD244.2-PE (cat#12-2441-82, eBioscience), Sca1-PC7, ckit-APC, and CD16/32 purified antibodies. Isotype control set constituted of Rat IgG1 Iso-FITC (cat#11-4301-82, eBioscience), Rat IgG2ak Iso-PE, Arm Ham IgG Iso-PE (cat#12-4888-81, eBioscience), Rat IgG2ak Iso-PerCP-Cy5.5 (cat#45-4321-80, eBioscience), Rat IgG2ak Iso-PC7 (cat#25-4321-82, eBioscience), Rat IgG2ak Iso-APC (cat#17-4031-82, eBioscience).

E. ROS measurement in BM cells

One million BM cells are incubated with 5 μ M CellROX probe (cat#C10422, Life Technologies) for 20 min at 37°C. Biotin-lineage antibody cocktail is then added and cells are incubated for 20 min at 4°C. Cells are washed with PBS and labeled with antibiotin-vioblu, Sca1-PE (cat#553336, eBioscience) and ckit-PC7 (cat#558163, eBioscience) antibodies for additional 20 min at 4°C. After wash with PBS, the cells are immediately analyzed by flow cytometry.

F. Progenitor cell assay

For CFC assay, 20,000 BM cells are seeded in Methocult™ GF M3434 (cat#03434, Stem Cell™ Technologies). For irradiated mice 40,000 cells are seeded for doses \leq 4 Gy and 100,000 cells for 8 Gy. Number of colonies is counted at day 10 with an optical microscope (Nikon Eclipse Ti-U).

G. Mouse sensitivity to induced stress

To test for the stress-induced sensitivity, survival of mice is followed after their irradiation at variable doses by the use of gamma irradiator at Xavier-Arnoz Hospital or after their injection with 150 mg/Kg of 5-FluoroUracil (5-FU) twice with a one week interval.

H. Statistical analysis

Data is presented either as the mean \pm S.E.M. or as the mean \pm standard deviation (SD). Statistical analysis is performed using the two-tailed student's t-test for comparison of two groups to determine the level of significance. Results are considered significant when p-value < 0.05 (*), more significant when p-value < 0.01 (**), and highly significant when p-value < 0.001 (***).

Chapter 3

RESULTS

I. Study of XPC expression in hematopoietic stem/progenitor cells (HSPC)

A. XPC is highly expressed in CD34⁺ cells

The XPC expression in mouse HSC and its downstream progenitor cells obtained by large-scale microarray analysis has been recently documented (Beerman et al., 2014). Interestingly, XPC was differentially expressed between the immature HSC and the downstream progenitor cells demonstrating a regulation of expression during differentiation. However, none of the previous studies reported the XPC expression in different human hematopoietic compartment. Therefore, XPC expression, at both the transcriptional and translational levels, has been investigated in stem/progenitor cell and compared to expression in the mature cell fraction based on the expression of the CD34 marker, CD34⁺ and CD34⁻ respectively. The analysis of XPC mRNA level showed a higher expression in CD34⁺ cells (3.1 ± 0.5 folds) in comparison to CD34⁻ cells (**Figure 31A**). After that, in order to study the protein expression of XPC in CD34⁺ and CD34⁻ fractions, we used as positive control skin-derived keratinocytes that are known to express XPC (Rezvani et al., 2010). We observed that all CD34⁺ cells expressed XPC whereas it is more inconsistently expressed in CD34⁻ cells (**Figure 31B**). Surprisingly, compared to keratinocytes, WB analysis on hematopoietic cells revealed a different pattern of expression. While the expected 125 kDa band is observed as in keratinocytes, additional bands with lower apparent molecular weight were observed in hematopoietic cells (about 100 and 110 kDa). The protein quantification, taking into consideration all the bands, shows significant higher expression of XPC protein (13.8 ± 8.4 folds) in CD34⁺ cells in comparison to CD34⁻ cells (**Figure 31C**). Further analysis of the three XPC bands shows that the 110 and 125 kDa bands expression is mostly linked to the immature state of the cells, CD34⁺ cells having 2.8 ± 1.0 and 5.1 ± 1.1 folds more expression than CD34⁻ respectively. In contrary, the 100 kDa band is less abundant in CD34⁺ cells than CD34⁻ cells by 0.48 ± 0.08 folds (**Figure 31D**). Hence, XPC is upregulated in CD34⁺ cells in comparison to CD34⁻ cells at both the transcriptional and the translational level.

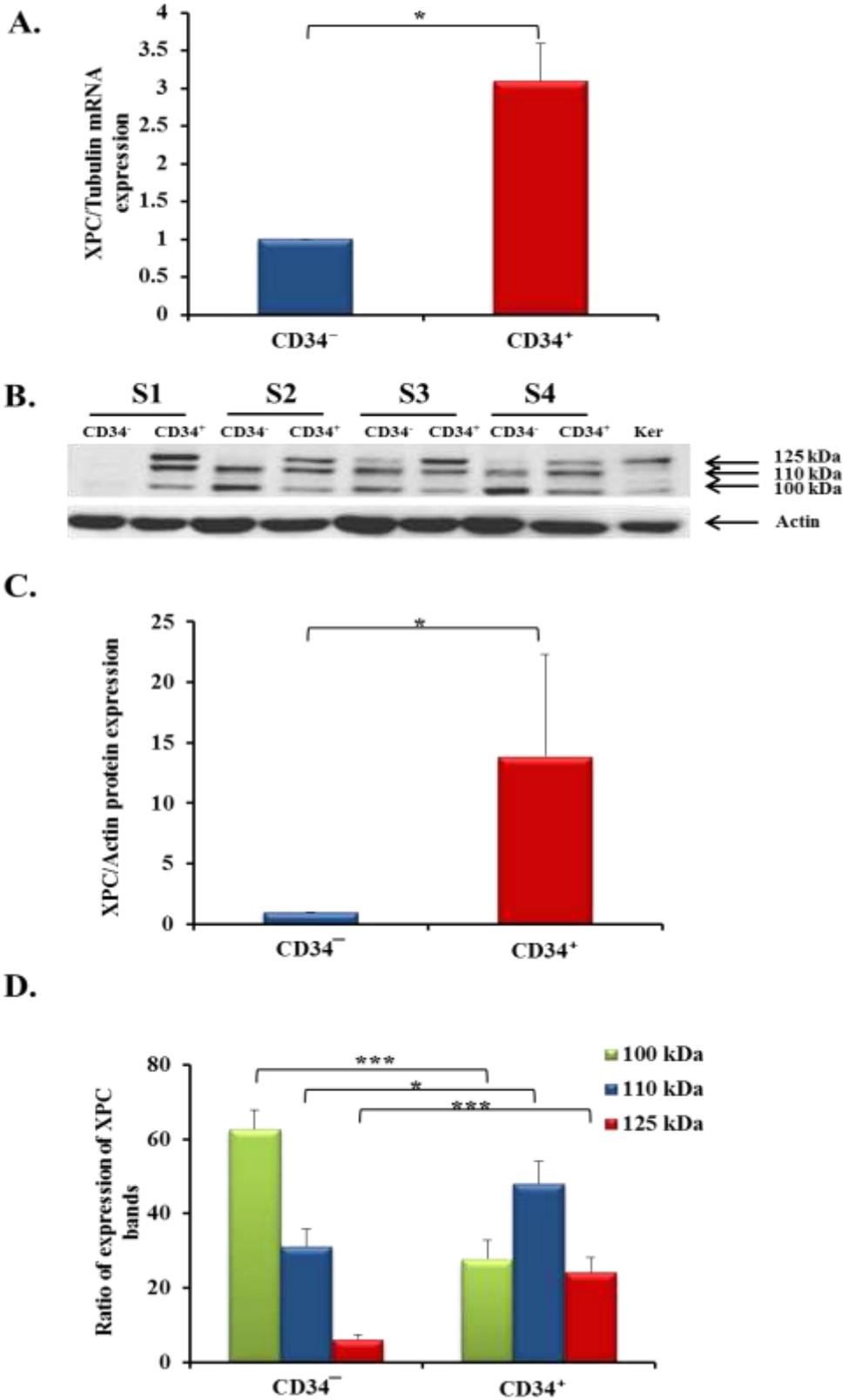


Figure 31. XPC expression in human CD34⁺ and CD34⁻ fractions. (A) RNA from UCB-derived CD34⁺ and CD34⁻ samples were subjected to Q-RT-PCR for assessment of XPC RNA expression in CD34⁺ and its CD34⁻ counterpart (n = 3). Values represent the average fold change of XPC expression in CD34⁺ cells, normalized to tubulin, and relative to CD34⁻ cells. (B) Total protein extracts from human CD34⁺ and its CD34⁻ counterpart were assessed for XPC protein expression by western blot. Actin was used as loading control. The arrowheads indicate the three XPC bands estimated at 100, 110 and 125 kDa. Keratinocytes from skin were used as positive control. S1, S2, S3, S4 represent four individual UCB samples. (C)

Protein quantification analysis of the WB shown in A (n = 12). Values represent the average fold change of XPC expression in CD34⁺ cells, normalized to actin, and relative to CD34⁻ cells. **(D)** Protein quantification analysis of each XPC band (n = 12). Values represent the ratio of each XPC band to total XPC bands in CD34⁺ and corresponding CD34⁻. Results are shown as the mean ± SEM. *p < 0.05, **p<0.01, ***p<0.001.

XPC expression has been further investigated in different subfractions of mature blood cells. To achieve that, monocytes, lymphocytes, and polynuclear cells were sorted from peripheral blood. XPC appears to be expressed exclusively in lymphocytes and not expressed in polynuclear cells and monocytes (**Figure 32**). Interestingly, XPC analysis shows inconsistent multi-band profiles in the lymphocytes similar to XPC profile in CD34 cells which might be due to alternative splice variants or post-transcriptional modifications or even degradation.

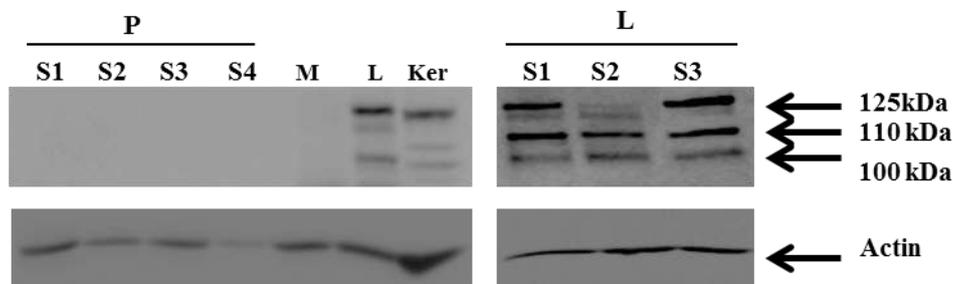


Figure 32. XPC expression in human mature blood cells. The XPC expression in monocytes, lymphocytes and polynuclear cells, purified by sorting from four human PB samples, was assessed by WB. β-actin was used as loading control. Keratinocyte cells were used as positive control. Symbols: *P*: Polynuclear cells; *L*: Lymphocytes; *S*: Sample; *M*: Monocytes; *Ker*: Keratinocytes.

However, to exclude unspecific recognition by antibody, which targets the C-terminal region, an anti-XPC antibody that recognizes the N-terminal region has been used. Both antibodies detect the same XPC bands (**Figure 33**).

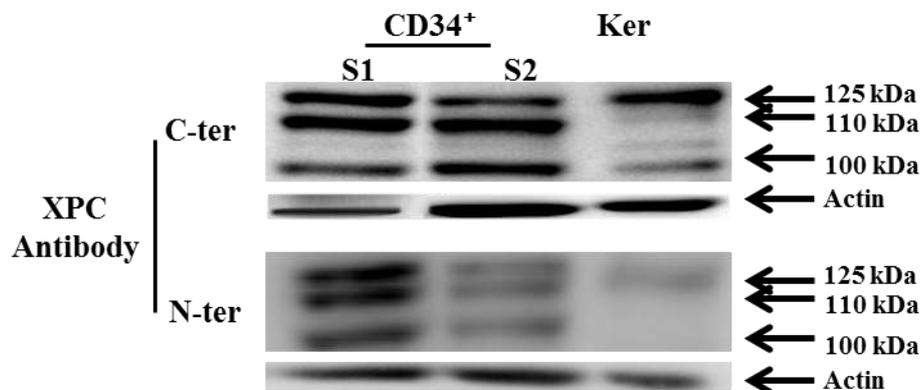


Figure 33. Investigation of XPC protein expression profile by two antibodies. Western blot of CD34⁺ cells isolated from 2 patients and analyzed simultaneously for XPC expression using both C-terminal anti-XPC antibody and N-terminal anti-XPC antibody. Keratinocyte was used as positive control.

B. XPC is expressed in leukemic cell lines

In humans, several pharmacogenomics studies have shown that *XPC* polymorphisms is associated with increased risk of leukemia and altered susceptibility to genotoxic effects of treatment (Guillem et al., 2010a; Strom et al., 2010). Thus, considering the data from normal hematopoietic cells we decided to investigate *XPC* expression in different cell lines representative of different lineages: from lymphoid (NALM6), myeloid (HL60, U937, THP1) and erythroleukemic (K562) origin (**Figure 34A**). Notably, the expression of *XPC* has been previously documented in leukemic cell lines at the transcriptional level but none at the protein level (Hsu et al., 2007). Our results showed that all the leukemic cell lines expressed *XPC* but, as showed before, with different profiles. Some lines mainly expressed the 125 kDa band (U937, NALM6, NB4 and K562), others expressed mainly the 110 kDa band (THP1), and other cell lines expressed both the 125 kDa and 110 kDa bands with equal intensity (HL60, KG1). Surprisingly, the AML-derived cells revealed an *XPC* protein expression in their cells while we previously found that the normal myeloid polynuclear cells sorted from the peripheral blood do not express *XPC*. In order to get a broader view of the myeloid cells and to determine whether expression profile was related to the differentiation status, we investigated *XPC* expression in a wide panel of acute myeloid leukemia (AML) cell lines classified following the French American British (FAB) subtypes (M0, M2, M3, M4, M5 and M7). Interestingly, we found that the highest molecular weight corresponding band is mainly expressed in M0/1 and in the M6/7 group FAB cell lines while the profile of *XPC* in M5 group FAB cell lines depends on the cell. This suggests that the *XPC* molecular weight is partially linked to the differentiation status of the cells but also due to other intrinsic mechanisms (**Figure 34B**). To exclude the fact that band appearance could be attributed to non-specific recognition we knockdown *XPC* through transduction of AML cell lines by a lentiviral vector carrying a shRNA directed against *XPC*. High inhibition of *XPC* expression (up to 99%) has been obtained in all cell lines and the knockdown affects all the different *XPC* forms confirming that the second and the third band observed are true *XPC* bands and not artifacts (**Figure 34C**).

Overall, the discrepancy between different bands might be explained by either alternative splice variants as suggested by Warrick et al. (Warrick et al., 2012) or by post-transcriptional modification such as phosphorylation (Matsuoka et al., 2007; Nguyen et al., 2010; Wu et al., 2006), sumoylation (Poulsen et al., 2013; Wang et al., 2005), and ubiquitylation (Lubin et al., 2014; Nospikel, 2011; Wang et al., 2007).

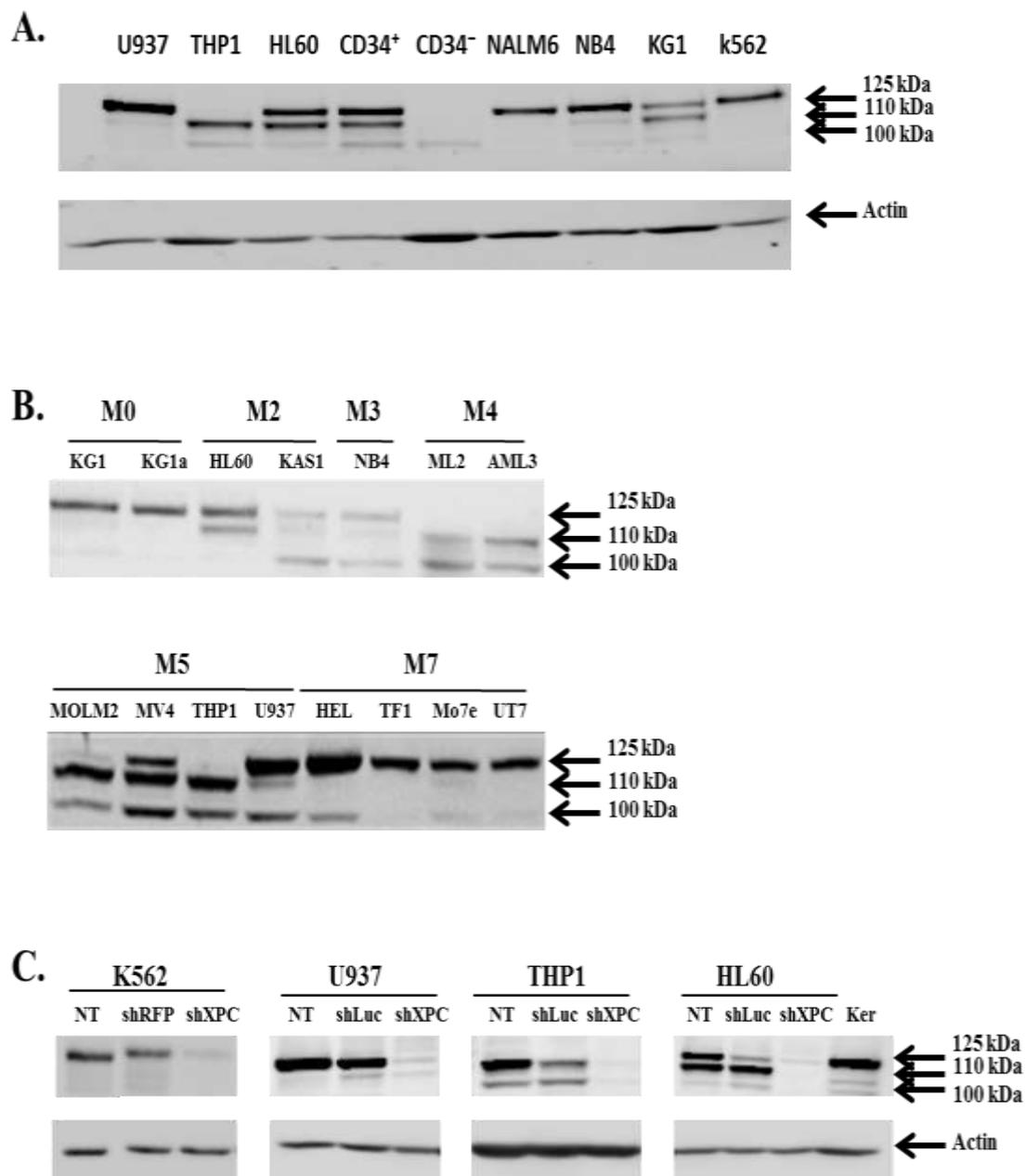


Figure 34. XPC expression in leukemic cell lines. (A) Western blot for different forms of XPC in hematopoietic cell lines and actin was used as control. (B) Western blot for AML cell lines belonging to different FAB subtypes M1-7. (C) Western blot of more than 90% transduced AML cell lines (K562, U937, THP1 and HL60) with shLuc/shRFP control vector or shXPC vector. *Symbol: NT: Non-transduced; S: sample.*

First, we intended to determine whether the extra XPC 110 and 100 kDa bands are characteristic of the hematopoietic cells and not keratinocytes. To do that, we performed protein analysis on keratinocyte from different individual samples. A unique XPC band has been observed in all keratinocyte samples, corresponding to 125 kDa band although it is possible to see extra but faint bands in samples S2, S4 and S8 (**Figure 35**). This confirms that the appearance of the three bands of XPC is particular to the hematopoietic cells.

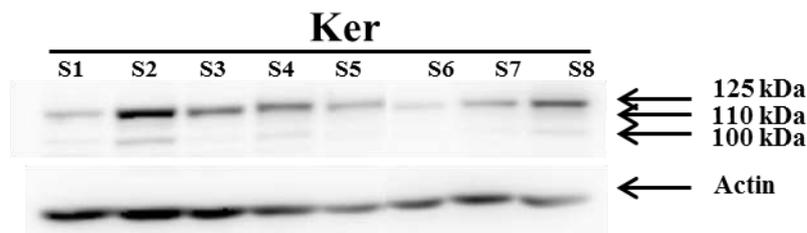


Figure 35. Investigation of XPC protein expression profile. Western blot of keratinocyte cells from 8 patients analyzed for XPC expression profile using actin as loading control.

In parallel, we tested for a possible alternative splicing. To achieve that, we used the HL60 cell line and perform RT-PCR. Four pairs of primers were designed to cover the whole coding sequence of *XPC* (**Figure 36A**). An alternative splicing should generate mRNA transcripts of different sizes. However, only one band has appeared for all primer pairs suggesting that the different protein weights are certainly not triggered by alternative splicing and highly suggests post-transcriptional modifications (**Figure 36B**).

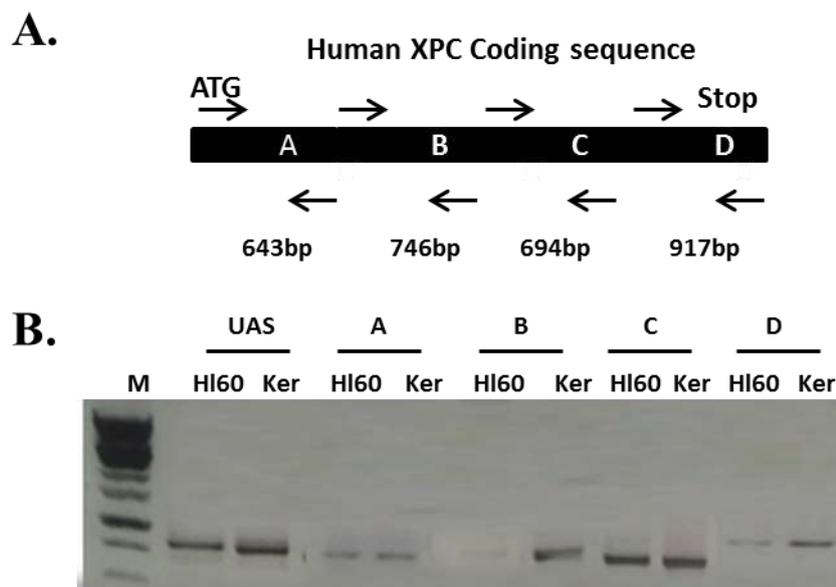


Figure 36. Investigation of XPC alternative splicing. (A) Schematic representation of the four primer couples used in the PCR reaction that were designed to span the XPC coding sequence. (B) Agarose gel results of amplified cDNA from HL60 and Ker cells by PCR using primer couples A-B-C-D. An additional condition using primers for UAS gene was included to serve as control. *Symbol: UAS: Upstream Activation Sequence; M: Marker; Ker Keratinocyte, A: region A of XPC gene; B: region B of XPC gene; C: region C of XPC gene; D: region D of XPC gene.*

C. XPC expression decreases throughout CD34⁺ culture

In order to better relate the XPC expression and the differentiation status, we performed *in vitro* culture of CD34⁺ cells using Stem Span medium supplemented with TPO, SCF, and Flt3L cytokines and also with SR1 and we followed the XPC expression. First of all, we have

found that the global XPC expression decreases with time (**Figure 37A-B**) by up to 88% after two weeks of culture. In parallel, the percentage of CD34⁺ cells was followed and used as a differentiation indicator. Data clearly show that the loss of CD34⁺ cells is accompanied by downregulation of XPC (**Figure 37C**). Interestingly, the expression of the different XPC protein bands was also modified according to the loss of CD34 expression. Results show decrease of the 125 kDa band (0.34 ± 0.10 folds) concomitant to increase of the 100 kDa band (1.9 ± 0.3 folds) after 14 days of culture (**Figure 37D**).

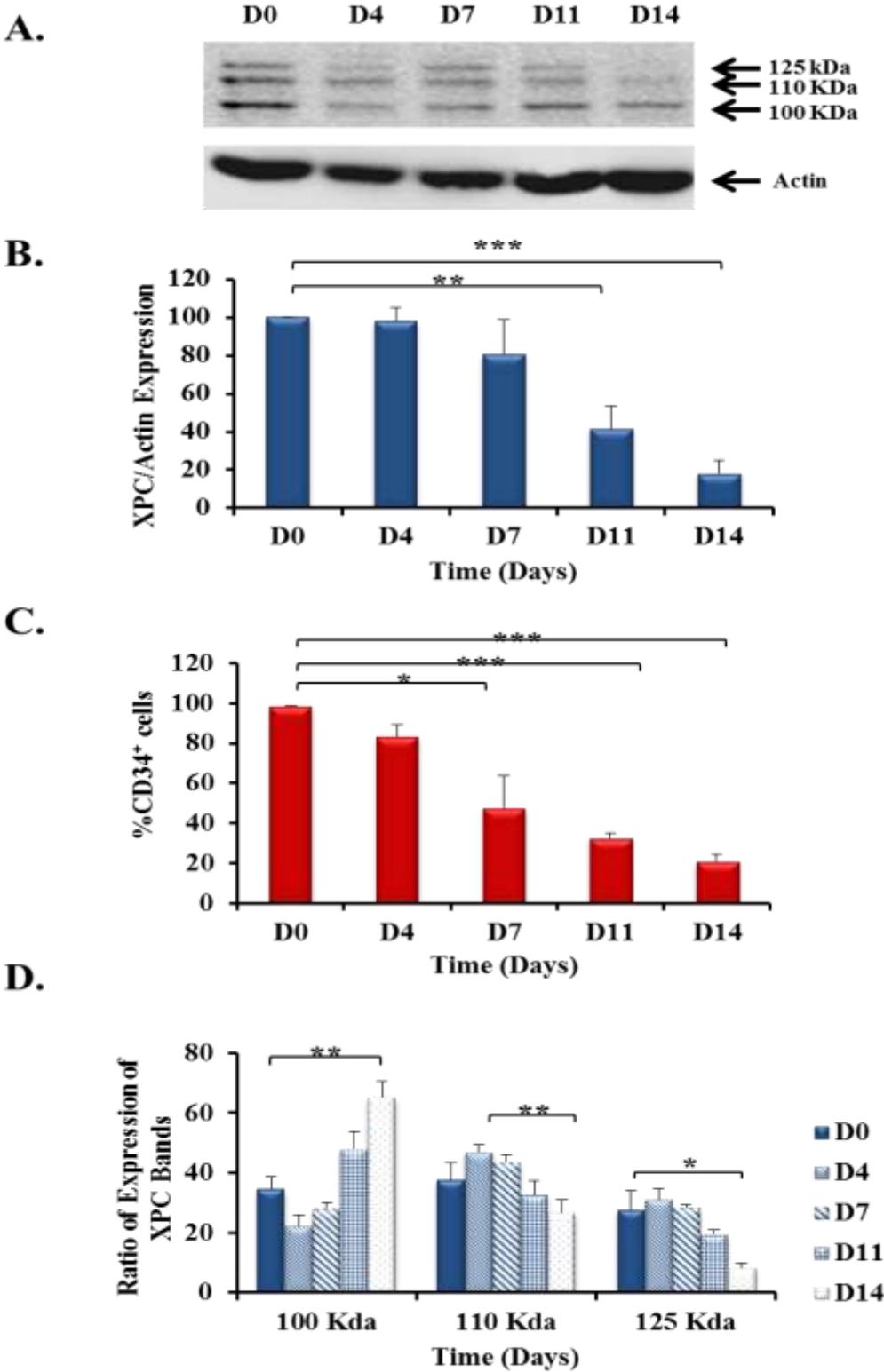


Figure 37. XPC expression during CD34⁺ liquid culture. (A) Western blot of CD34⁺ cells collected at different days during the culture was performed to reveal the expression of XPC. (B) Protein quantification of the western blot in (A) is represented by the ratio of XPC expression to actin expression. (C) Percentage of CD34⁺ cells were analyzed by FACS at different days of liquid culture. (D) Protein quantification of 125, 110 and KDa XPC bands in (A) is represented in form of ratio of XPC expression of each band to the total XPC expression at the different days of culture. Results are shown as the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

To further confirm that the residual expression of XPC is restricted to CD34⁺ cells in culture, CD34⁺ cells were cultured until day 11 and sorted based on the CD34 expression. Data revealed a quasi-exclusive XPC expression in CD34⁺ cells (**Figure 38A**). Quantification of the total bands showed a much higher expression of XPC (42 ± 30 folds) in purified CD34⁺ compared to CD34⁻ fraction (**Figure 38B**).

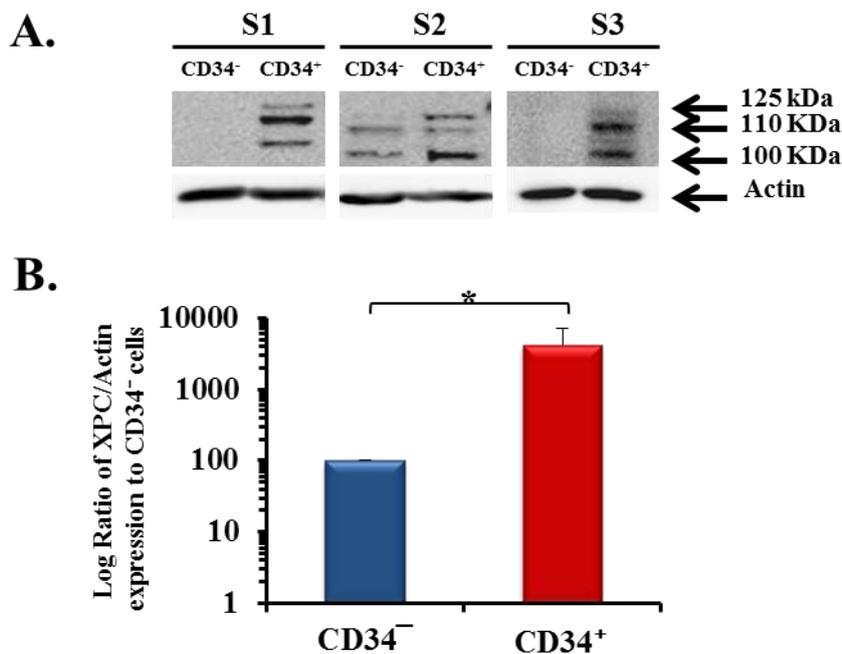


Figure 38. XPC expression in sorted CD34⁺ cells after liquid culture. (A) Western blot was performed on sorted CD34⁺ (95.2 ± 2.3 %) and CD34⁻ (100%) fractions originating from 11-days cultured CD34⁺ cells. (B) Protein quantification of the western blot in (A) is represented in form of ratio of normalized XPC/Actin expression in CD34⁺ cells and relative to CD34⁻ cells. Results are shown as the mean ± SEM. *p < 0.05. Symbols: S: sample.

Overall, our data have evidenced the existence different levels of expression as well as different expression profiles of the XPC protein in hematopoietic cells. Investigating the XPC expression profile, we have showed that the 125 kDa band, corresponding to the highest molecular weights, are mainly expressed in immature cells (CD34⁺ versus CD34⁻; AML M0/1 versus AML M3/4/5). Data highly suggest post-transcriptional modifications of the protein, which could be then associated with either stability or/and activity of the protein.

Consistently, we showed decrease of XPC protein expression during CD34⁺ differentiation in culture. The higher expression of XPC in CD34⁺ fraction compared to the more mature CD34⁻ fraction suggests that XPC might be related to the maintenance of the CD34⁺ cells. Therefore, our next step was to study the functional role of XPC in CD34⁺ cells.

II. Study of the impact of the *XPC* silencing in human HSPC

A. *XPC* silencing does not affect proliferation of CD34-derived cells in culture

In order to study whether *XPC* might affect hematopoietic cell behavior, we attempted to knockdown its expression in CD34⁺ cells using lentiviral vectors that carry interfering RNA targeting *XPC*. To determine the inhibition efficacy at a low copy number of the lentiviral vector, cells were transduced at 20 to 30 % and sorted based on GFP expression (**Figure 39A-B**). WB analysis shows that all *XPC* bands were extinguished (71 ± 3.5 % reduction) demonstrating the efficiency of the vector in CD34 cells (**Figure 39C-D**).

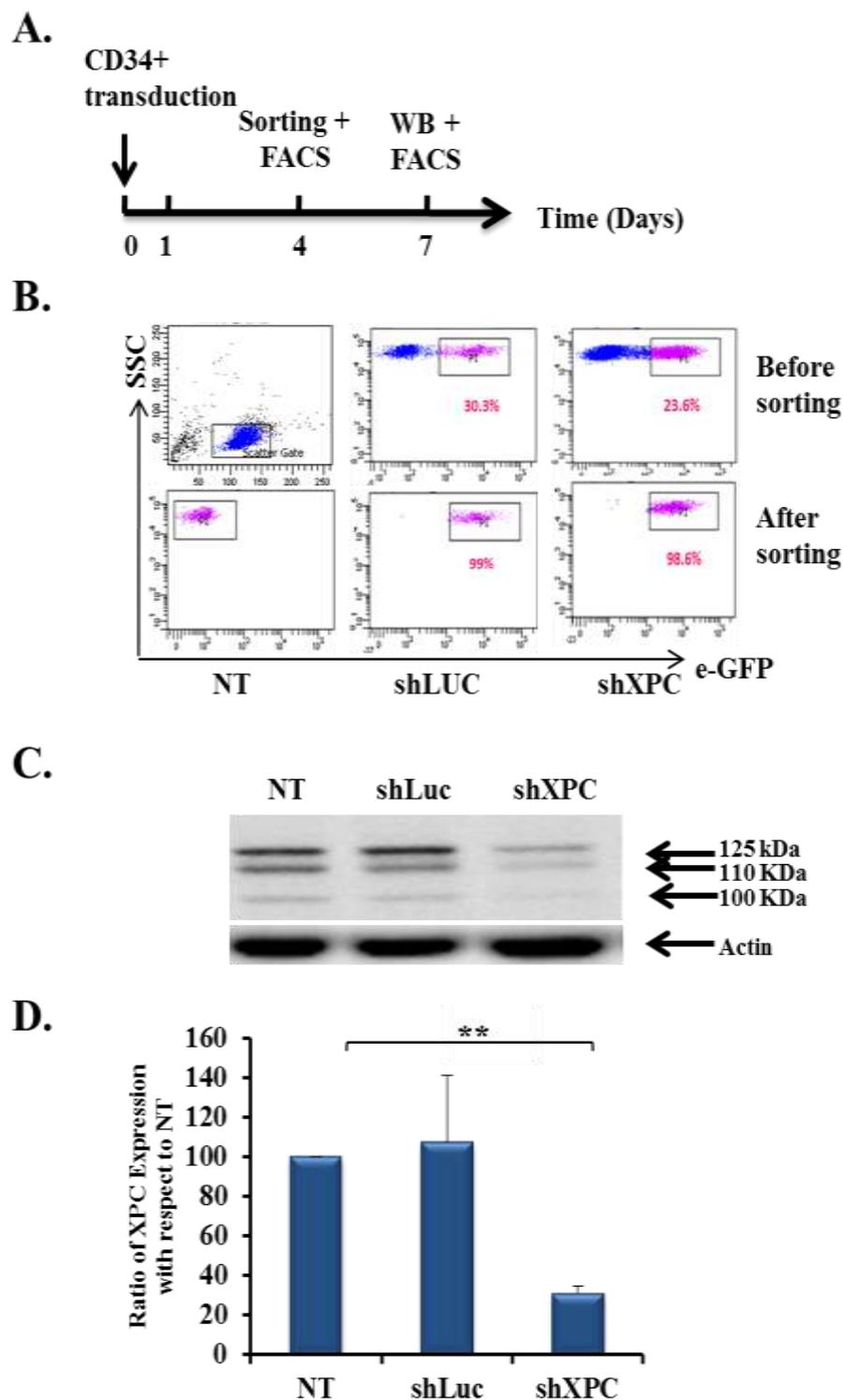


Figure 39. Validation of the XPC knockdown efficiency in CD34⁺ cells by shXPC-GFP lentiviral transduction method. (A) Schematic representation showing the experimental planning to check whether XPC silencing affects the stability of CD34⁺-derived cells. CD34⁺ cells were transduced at M.O.I = 100 with either the shXPC vector or the control shLuc vector. Cells were then seeded in liquid culture (Stem Span + cytokines SCF, Flt3L, TPO). (B) FACS data showing efficiency of sorting of pure GFP⁺ cells from transduced CD34⁺ cells with shLuc or shXPC vectors after four days of liquid culture. (C) Representative western blot analysis of XPC expression of sorted GFP⁺ shXPC and shLuc transduced cells after seven days of liquid culture. Cells at day seven consisted of >96% CD34⁺ cells and 87% GFP⁺ cells. (D) Protein quantification of XPC expression in WB of (C) and represented as normalized XPC/Actin expression and relative to NT. (n = 3 independent experiments) Results are shown as the mean ± SEM. **p < 0.001. Symbols: WB: western blot.

The effect of *XPC* KD on the maintenance of CD34⁺ cells has been first investigated in liquid culture (**Figure 40A**). The proliferation of cells was followed by numeration of control (shLuc) and shXPC-transduced cells during the culture (**Figure 40B**). Data show that both the proliferative and differentiation potential of CD34⁺ cells has not been affected by *XPC* KD. The amount of CD34⁺ cells and GFP⁺ cells remained comparable in both conditions (**Figure 40C-D**).

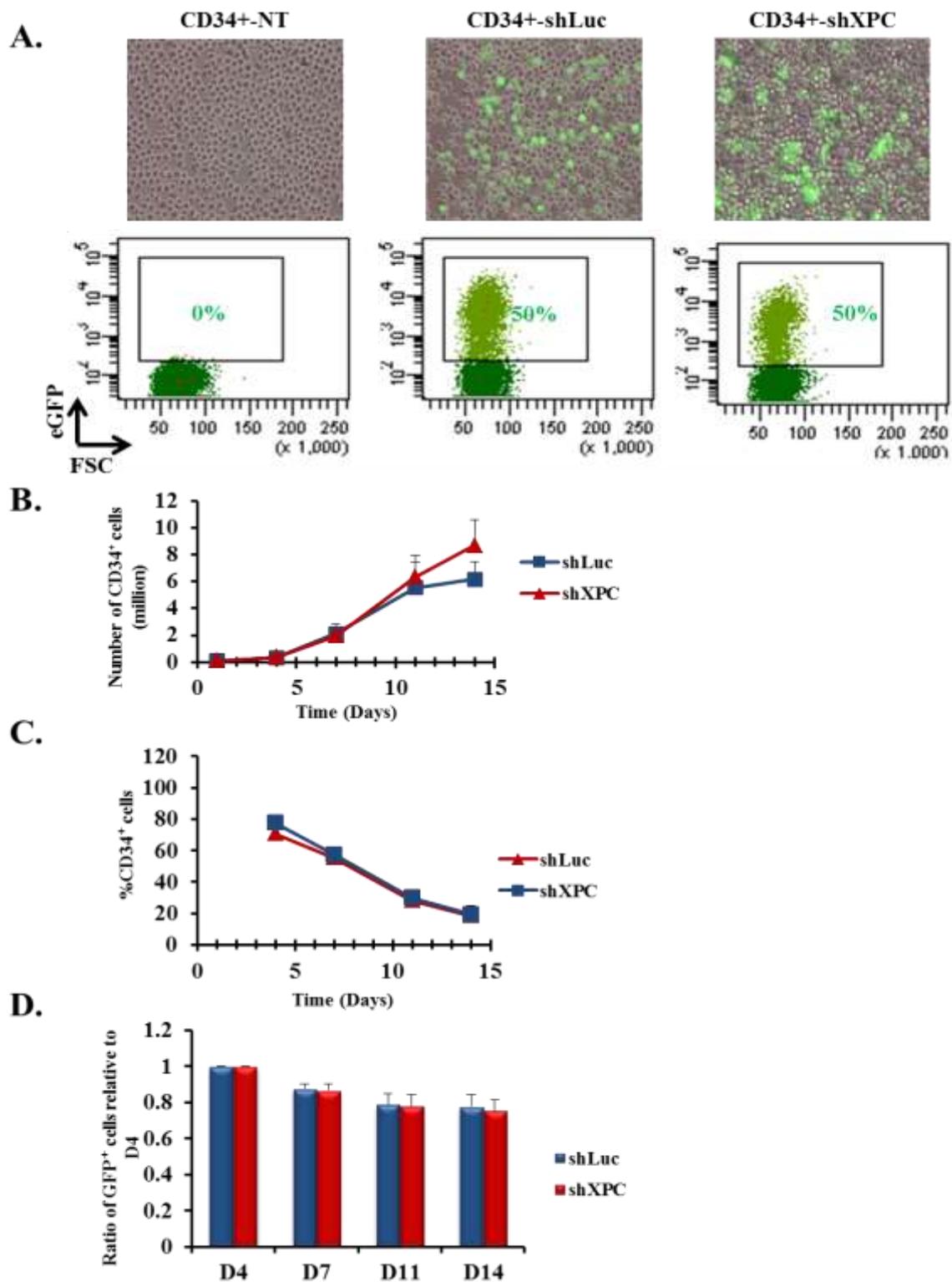


Figure 40. Effect of *XPC* silencing on CD34⁺ derived cells. (A) Fluorescent microscopy images of CD34⁺ cells transduced with shLuc-GFP control vector or shXPC-GFP vector (top image). Corresponding FACS images of CD34⁺ cells transduced with shLuc-GFP and shXPC-GFP vector showing about 50% transduction efficiency at day 4. (B) Follow-up of proliferation of CD34⁺ cells transduced with shLuc and shXPC vectors (n = 3). (C) Follow-up of amount of CD34⁺ cells determined by FACS after liquid culture of cells transduced with shLuc and shXPC vectors (n = 4). (D) Follow-up of amount of GFP⁺ cells during liquid culture of CD34⁺ cells transduced with shLuc and shXPC vectors (n = 6).

B. *XPC* silencing does not affect committed progenitor cells

The function of CD34⁺ cells can be assessed by their ability to produce hematopoietic progenitors. In order to investigate whether *XPC* KD can affect the amount of progenitors produced by the transduced CD34⁺ cells, sh*XPC*- and shLuc-transduced CD34⁺ cells were seeded in semi-solid methylcellulose medium. Data did not show any significant difference between *XPC* KD cells and control in terms of percentage of expressing cells suggesting that *XPC* is not required for progenitor repopulating ability (**Figure 41A**). In addition, no difference has been observed between myeloid or erythroid progenitors (**Figure 41B**). Thus, *XPC* KD does not seem to impact CD34⁺ differentiation.

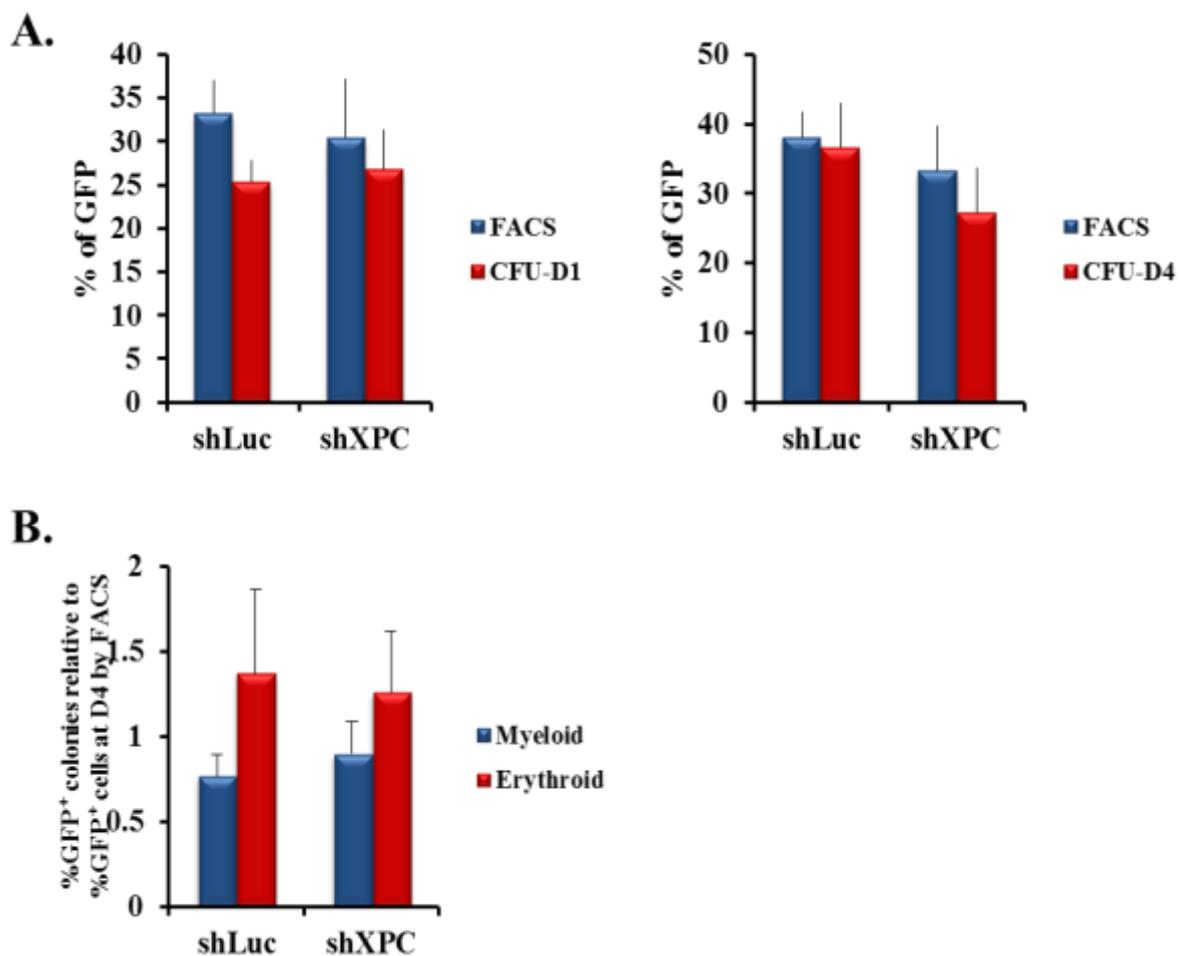


Figure 41. Role of *XPC* in progenitor repopulating ability of CD34⁺ cells. (A) Graph representing the percentage of GFP at day 4 of culture of transduced CD34⁺ cells with sh*XPC* or shLuc (control) determined by FACS from one side and the percentage of GFP⁺ colonies produced by sh*XPC* and shLuc transduced CD34⁺ cells seeded in methylcellulose after 1 day of transduction (n=8) and after 4 days of transduction (n=8). (B) The percentage ratio of myeloid and erythroid GFP⁺ colonies has been determined by CFC assay relative to %GFP⁺ cells determined by FACS at day 4 of transduction in shLuc- and sh*XPC*-transduced CD34⁺ seeded cells. Symbols: *BFU-E*: Burst forming unit-erythroid; *CFU-G/M*: colony forming unit-granulocyte/macrophage.

C. *XPC* silencing affect stem cells

1. *XPC* is crucial *in vivo* to sustain human hematopoiesis

In order to test the role of *XPC* on stem cell maintenance, it was essential to perform *in vivo* experiments. Therefore, xenotransplantation of CD34⁺ cells transduced with the sh*XPC* vectors were performed into immune-deficient NOD/SCID mice. The human cells were followed over the time by the percentage of CD45⁺ cells in bone marrow aspirates and the transduced cells by the GFP-expression (**Figure 42A**). Our data showed decreased percentage of GFP⁺ cells with time in *XPC* KD cells compared to the control, thus indicating depletion of CD34⁺ cells *in vivo* and suggesting a putative role of *XPC* in the stem cell maintenance or proliferation of the committed progenitors (**Figure 42B**). Then, to demonstrate that the decrease was not due to a change in the distribution of cells in the different hematopoietic tissues, we checked the engraftment of the transduced cells in the BM, PB, spleen and thymus of these mice after 18 weeks of transplantation. Thus, we noticed a similar decrease in engraftment in each of the different tissues (**Figure 42C**).

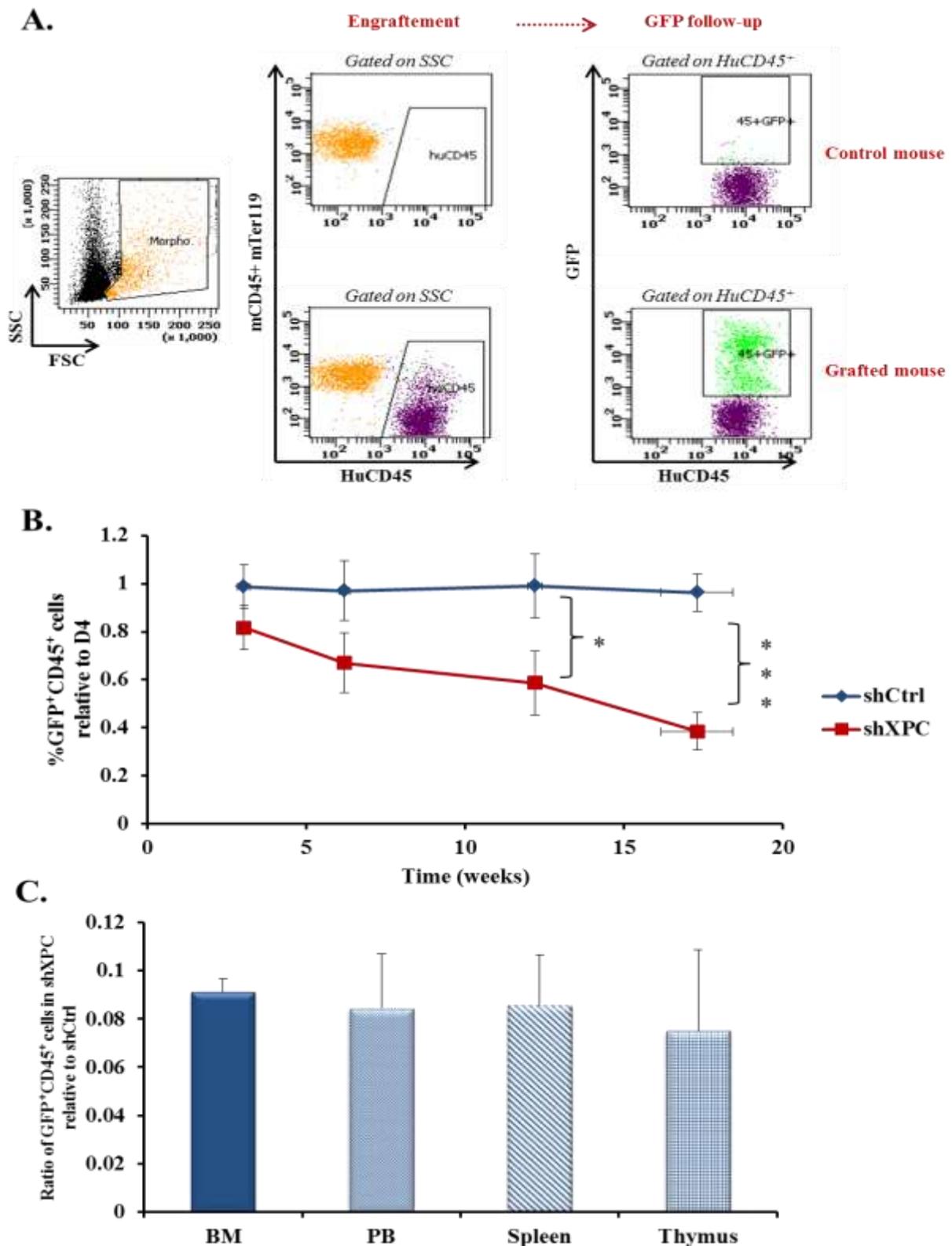


Figure 42. XPC effect on human cell engraftment. (A) FACS image showing gating technique to follow-up GFP percentage in NSG-transplanted human CD45⁺ cells. (B) Fate of shXPC- or shCtrl- transduced CD34⁺ cells engrafted in NSG mice is assessed for by the follow-up of the kinetics of HuCD45⁺GFP⁺ cells amount at different time points (3±0.2, 6.2±0.17, 12.2±0.24 and 17.3±1.13 weeks) of engraftment by aspirating bone marrow from primary mice at each time point and analyzing it (n=5 experiments; 13 NT mice, 17 shCtrl mice, 16 shXPC mice). (C) Histogram showing variation in ratio of % of HuCD45⁺GFP⁺ cells in shXPC relative to shCtrl in different organs (spleen, thymus, PB, BM) obtained from primary NSG recipient mice after 18 weeks of transplantation (n=5). Results are shown as the mean ± SEM. *p < 0.05, ***p < 0.001.

2. *XPC* silencing in various lineages

a. *XPC* silencing affects erythroid reconstitution

In order to determine whether *XPC* might affect hematopoietic lineages differently we followed the expression of the GFP in the different subpopulation of huCD45+ cells. First we analyzed the kinetics in erythroid CD36⁺ cells after 3, 6, 12, 18 weeks (**Figure 43A**). It has to be noted that erythroid fraction is highly represented at 3 weeks and then decline. We also observed in some mice an increased percentage of CD36⁺ cells after 18-24 weeks. Regarding the GFP, we have detected a gradual decrease in the percentage of GFP⁺ cells relative to day 4 with time (3 to 18 weeks) (**Figure 43B**). In the more mature cells characterized by the expression of GPA we also obtained a highly significant decrease in the ratio of GFP⁺ cells relative to day 4 (**Figure 43C**).

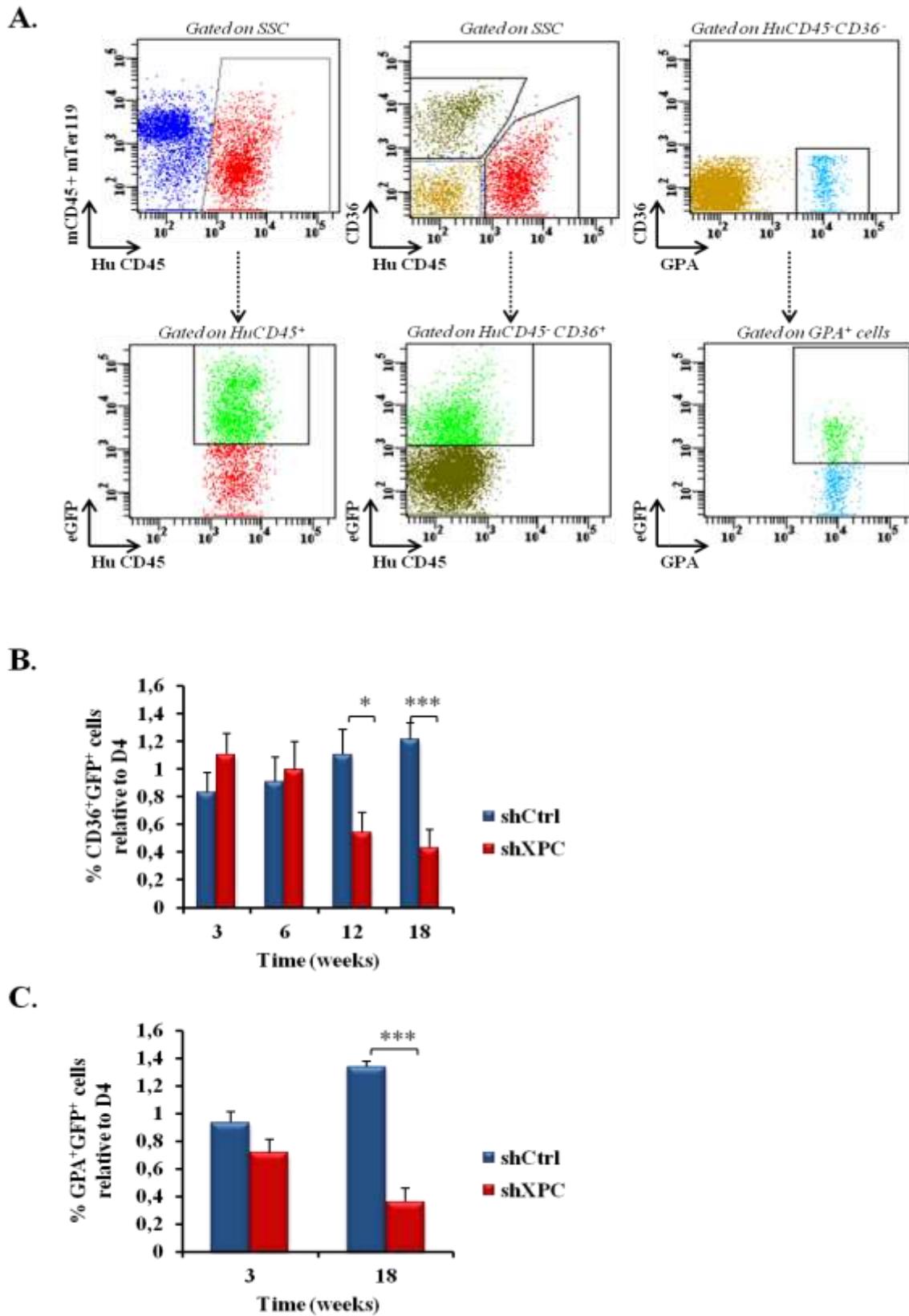


Figure 43. Effect of XPC knockdown on erythroid reconstitution. (A) FACS image showing gating technique to detect erythroid cells in transplanted NSG mice. (B) Histogram showing variation in ratio of percentage of CD36⁺GFP⁺ cells in shXPC relative to shCtrl after 3-6-12-18 weeks of transplantation (7 shCtrl mice, 9 shXPC mice). (C) Histogram showing variation in ratio of % of GPA⁺GFP⁺ cells in shXPC relative to shCtrl after 3 and 18 weeks of transplantation (5 shCtrl mice, 12 shXPC mice). Results are shown as the mean ± SEM. *p < 0.05, ***p < 0.001.

b. *XPC* silencing affects myeloid reconstitution

In order to investigate whether *XPC* KD impact the myeloid lineage, we checked the amount of human CD33⁺ and CD11b⁺CD14⁺ cells in the BM of our mice 18 weeks post transplantation (**Figure 44A**). We found that the ratio of CD33⁺GFP⁺ cells relative to day 4 with time decreased significantly after 18 weeks of transplantation in sh*XPC*-transduced mice in comparison to shCtrl-transduced mice (**Figure 44B**). Similarly, the ratio of CD11b⁺CD14⁺GFP⁺ cells relative to day 4 decreased significantly after 18 weeks in sh*XPC*-transduced mice in comparison to shCtrl-transduced mice (**Figure 44C**).

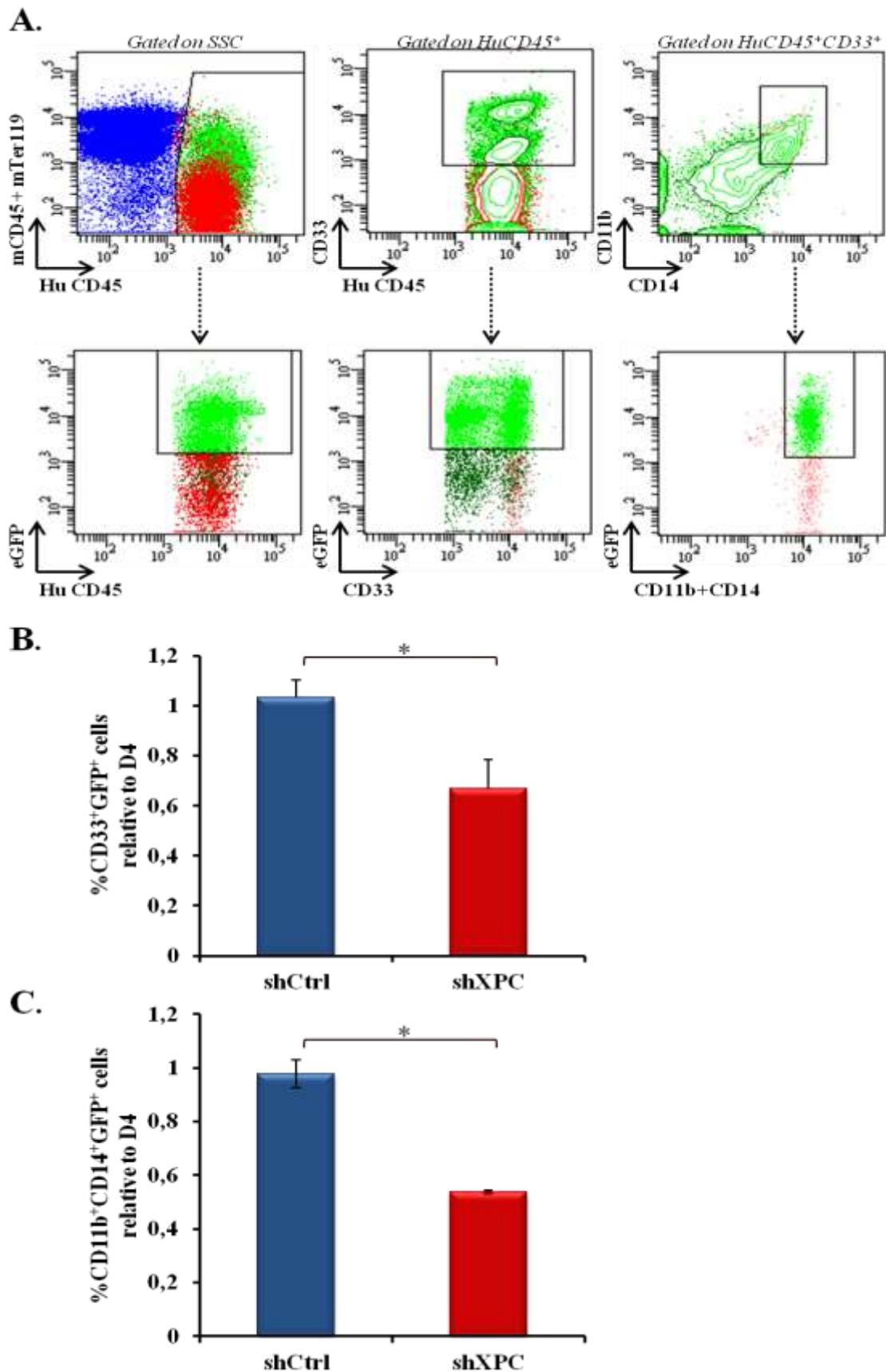


Figure 44. Effect of *XPC* knockdown on myeloid reconstitution. (A) FACS image showing gating technique to detect myeloid cells in transplanted NSG mice. (B) Histogram showing variation in ratio of % of CD33⁺GFP⁺ cells in shXPC relative to shCtrl after 3-6-12-18 weeks of transplantation (6 shLuc mice, 4 shXPC mice). (C) Histogram showing variation in ratio of % of CD11b⁺CD14⁺GFP⁺ cells in shXPC relative to shCtrl after 3-6-12-18 weeks of transplantation (5 shLuc mice, 6 shXPC mice). Results are shown as the mean ± SEM. *p < 0.05.

c. *XPC* silencing affects lymphoid reconstitution

In order to investigate whether *XPC* KD impact on the lymphoid lineage, we checked the amount of human CD19⁺ and CD3⁺ cells, representing B- and T-lymphocytes respectively, in the BM of our mice 18 weeks post transplantation (**Figure 45A**). The detection of the %CD19⁺GFP⁺ cells 18 weeks have revealed strong decrease in sh*XPC*-transduced mice compared to shCtrl-transduced mice (**Figure 45B**). Similarly, the detection of the %CD3⁺GFP⁺ cells 18 weeks have revealed strong decrease in sh*XPC*-transduced mice compared to shCtrl-transduced mice (**Figure 45C**).

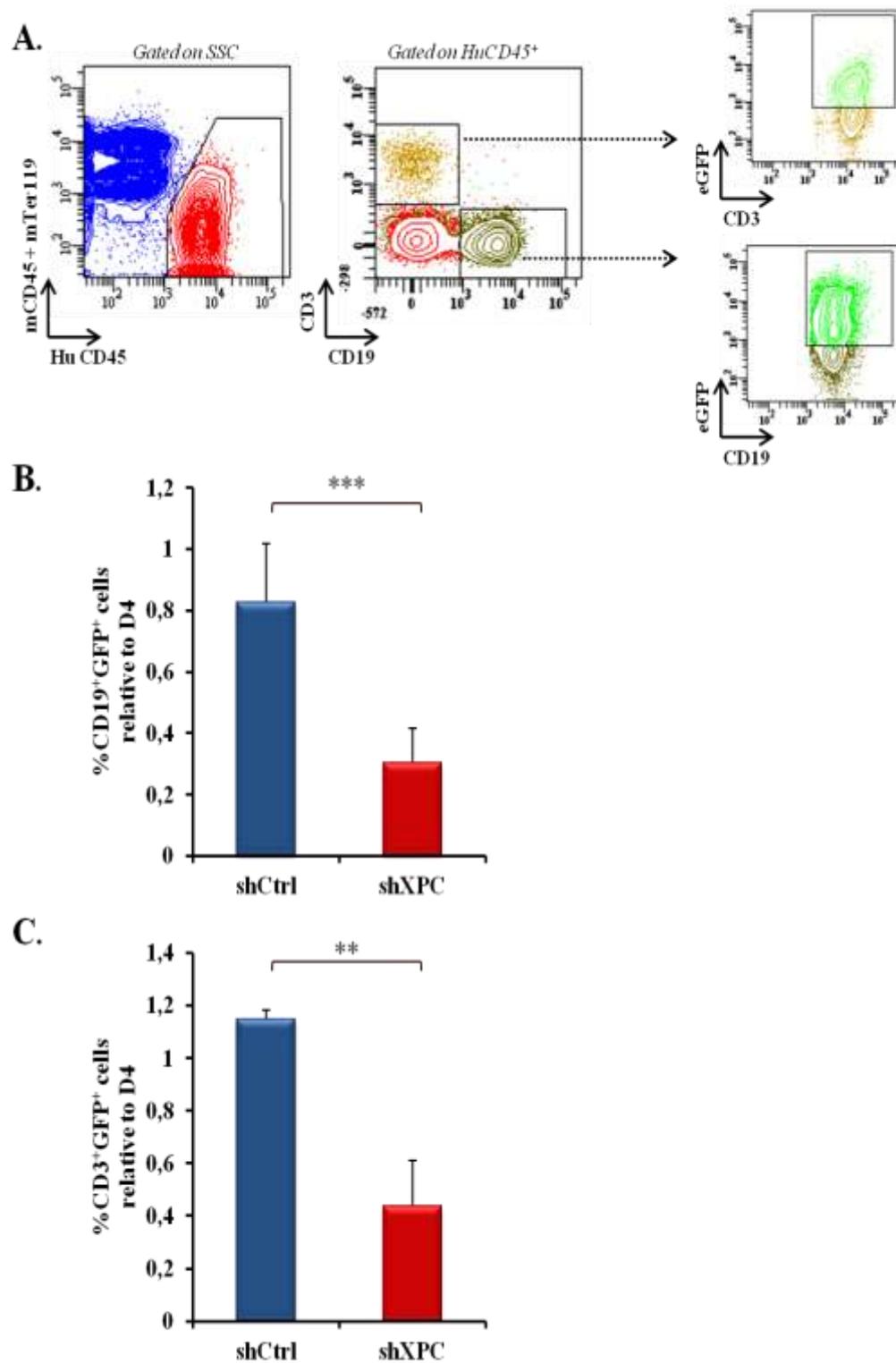


Figure 45. Effect of XPC knockdown on lymphoid reconstitution. (A) FACS image showing gating technique to detect lymphoid cells in transplanted NSG mice. (B) Histogram showing variation in ratio of % of CD19⁺GFP⁺ cells (left) and % of CD3⁺GFP⁺ cells (right) in shXPC relative to shCtrl after 18 weeks of transplantation (n = 4 experiments, 14 shCtrl mice, 11 shXPC mice). Results are shown as the mean ± SEM. **p < 0.01, ***p < 0.001. (C) Histogram showing variation in ratio of % of CD3⁺GFP⁺ cells in shXPC relative to shCtrl after 18 weeks of transplantation (n = 2 experiments, 4 shCtrl mice, 7 shXPC mice). Results are shown as the mean ± SEM. **p < 0.01, ***p < 0.001.

3. *XPC* KD affects maintenance of stem cells

To explain the decrease in mature lineage we could hypothesize similar general mechanism in all the lineages or impact on the stem cell or early progenitors. To undercover this we determine the percentage of GFP cells within stem cell fractions according to the expression of various markers that are CD34, CD38, CD45RA. Thus, we attempted to check for the percentages of the stem cells subpopulations, ranging from the most immature stem cell population identified as Lin⁻CD34⁺CD38⁻CD45RA⁻ to the more mature Lin⁻ cells, present in the bone marrow of these mice 18 weeks post transplant (**Figure 46A**).

The detection of the %GFP⁺ cells in the BM cell populations following different degrees of maturity has revealed a strong decrease of GFP in *XPC* KD cells compared to shCtrl-transduced populations. Interestingly, all the stem cell subpopulations showed significant decrease in %GFP in sh*XPC*-transduced mice compared to shCtrl-transduced mice. Therefore *XPC* KD affects the stem cell maintenance in general and affects the Lin⁻CD34⁺CD38⁻ population of cells in particular (**Figure 46B**).

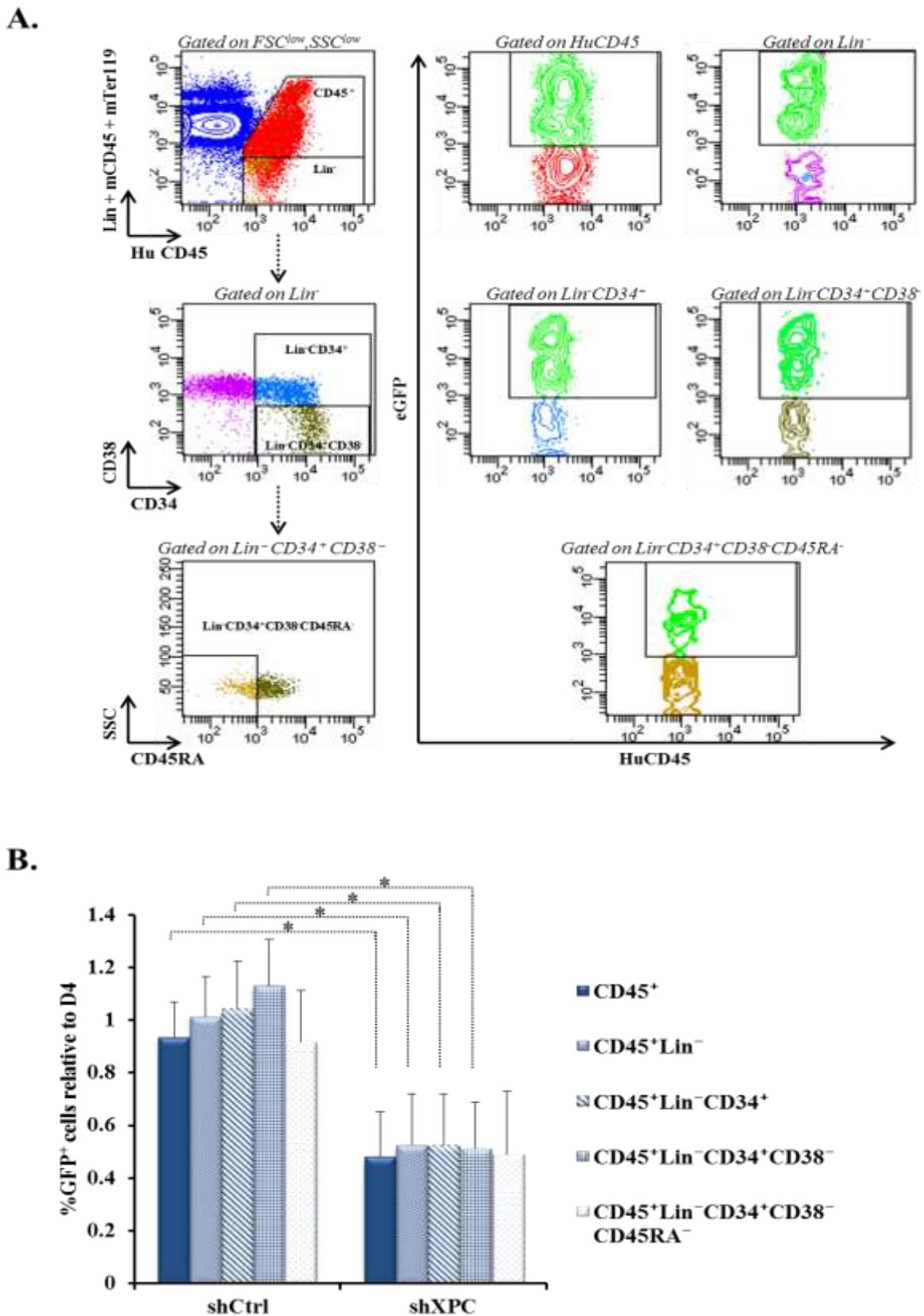


Figure 46. Effect of the XPC knockdown on stem cell compartments. (A) FACS image showing gating technique to detect different stem cell compartments in transplanted NSG mice. **(B)** Histogram showing the percentage of GFP⁺ cells relative to day 4 in different compartments 18 weeks after transplantation. Noteworthy, only FSC^{lo}SSC^{lo}huCD45⁺ cells were selected to gate for stem cells (7 shLuc mice, 5 shXPC mice). Only mice in which the more immature fraction (CD45RA⁻) provide enough events were selected (n = 3 experiments). Results are shown as the mean ± SEM. *p < 0.05.

4. *XPC* KD is crucial for long-term multilineage reconstitution

To confirm that long-term engraftment is affected by *XPC* KD and to address its impact on the self-renewal capacity of human HSPCs, primary BM cells have been transplanted in secondary recipients and GFP expression determined in human cells. Data show remarkable decrease ($76.7\% \pm 11.3$) in the percentage of engrafted sh*XPC*-transduced $CD34^+$ cells whereas not or few decrease has been noticed for control cells (**Figure 47**). The further reduction in the percentage of *XPC* KD cells observed at 18 weeks postsecondary transplantation as compared to the level obtained at 18 weeks postprimary transplantation strongly demonstrates that the maintenance of human long-term repopulating cells with self-renewing ability is dependent on *XPC*.

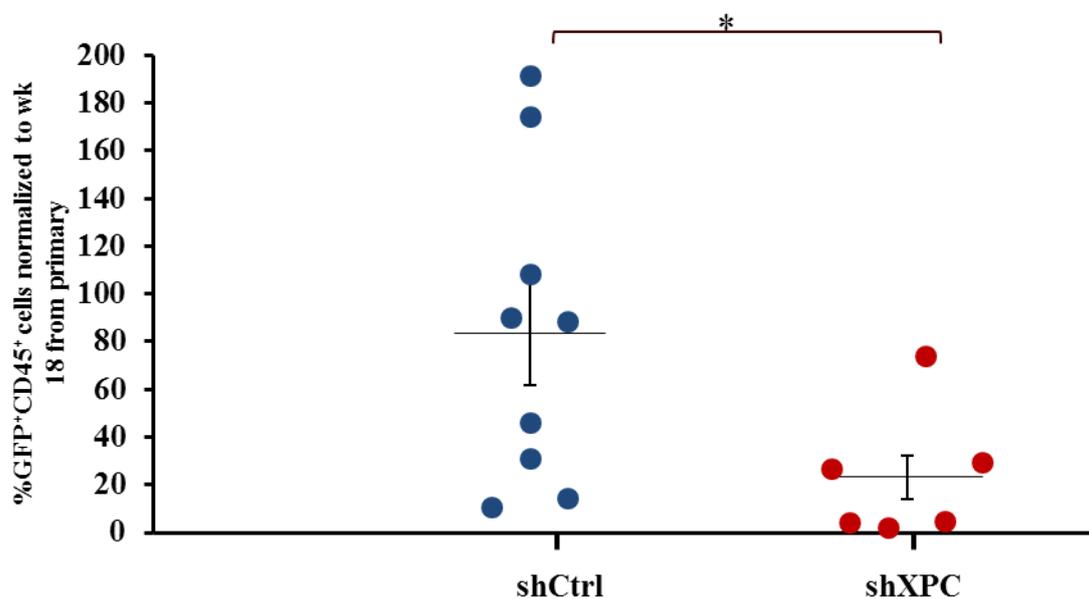


Figure 47. *XPC* effect on human cell engraftment in secondary mice. Dot plot of the ratio of $HuCD45^+GFP^+$ cells in secondary mice relative to primary mice showing fate of $HuCD45^+GFP^+$ cells in secondary mice after 18 weeks of transplant (9 shCtrl mice, 6 sh*XPC* mice). Results are shown as the mean \pm SEM. * $p < 0.05$.

D. *XPC* KD does not modulate ROS level in Lin^-CD34^+ cells

Studies have showed that *XPC* is implicated in redox homeostasis and prevents oxidative DNA damage (Melis et al., 2011, 2013a; Rezvani et al., 2011c). Indeed, the loss of *XPC* in keratinocytes triggers increased ROS production. To further explain the decline seen following the KD of *XPC*, we hypothesize an increase in the oxidative stress. Bone marrow cells from the mice were harvested 18 weeks post-transplantation and hematopoietic stem/progenitor cells labeled using Lineage-depletion cocktail, anti- $CD34$ and the CellROX probe which detected a wide range of ROS (**Figure 48A**).

Our data did not reveal any significant difference in the oxidative stress in Lin⁻CD34⁺ cells obtained from both shLuc- and shXPC-transduced mice (**Figure 48B**). Therefore the depletion of shXPC-transduced CD34⁺ cells in NSG mice is certainly not due to oxidative stress.

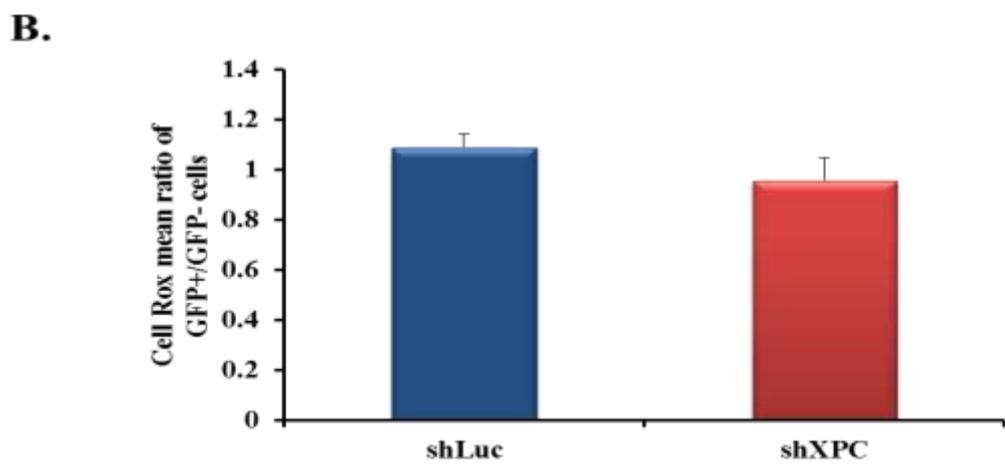
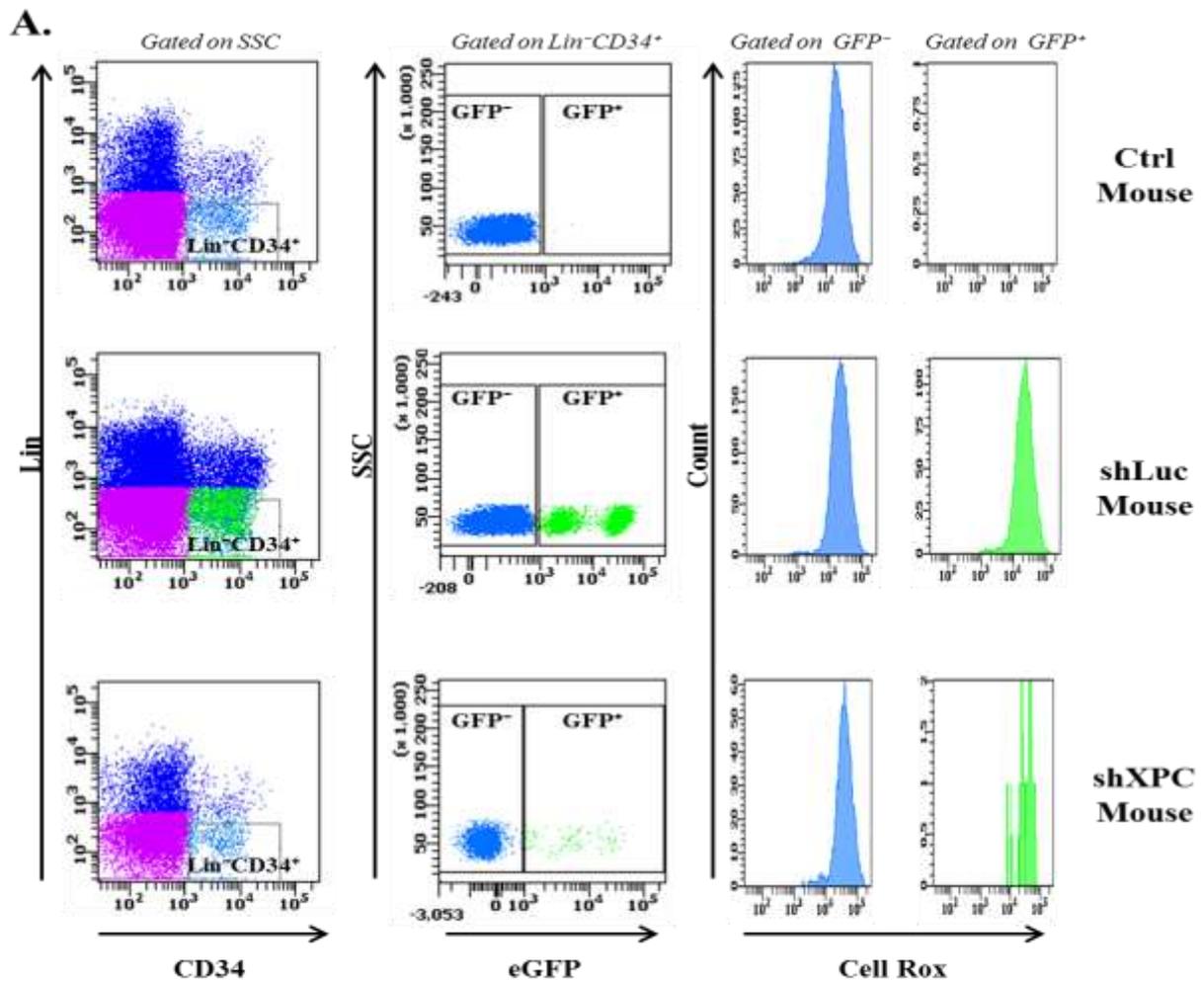


Figure 48. Effect of XPC knockdown on oxidative stress. (A) FACS image showing gating used to detect ROS level in Lin⁻CD34⁺ cells from GFP⁺ or GFP⁻ fraction in transplanted NSG mice. (B) Histogram showing variation in the ratio of mean

fluorescence of CellROX in GFP⁺ cells relative to GFP⁻ cells in Lin⁻CD34⁺ cells from bone marrow. The result represents the mean of 4 experiments (12 shLuc mice, 5 shXPC mice).

In conclusion, we have showed *in vitro* that *XPC* KD neither affect the proliferation and differentiation of the CD34-derived cells in liquid culture nor the ability of progenitor cells. However, we showed *in vivo* that *XPC* is involved in long-term hematopoietic capacity. All together, these data suggest a role of *XPC* in stem cell compared to committed/mature cells. However, it is likely wise to think that *in vitro* culture conditions might protect the cells from oxidative stress, and consequently might prevent their loss of function. However, *in vivo* loss of stem cells following *XPC* KD does not seem to be related to increase ROS levels. Further investigation should be held at the molecular level in order to find the pathways leading to the loss of long-term hematopoietic ability (apoptosis-senescence...).

III. Study of the hematopoiesis in the murine *Xpc* KO

It has been documented that NER is involved in HSC maintenance with age in the *xpd* KO model where an age-dependent decline in HSC activity was observed (Rossi et al., 2007a). However, it has not been demonstrated whether the GGR or the TCR is the major pathway involved in hematopoiesis. Recent paper from the same research team tend to support the idea that XPC, key factor of GGR, is modulated during differentiation of mouse HSC into progenitors (Beerman et al., 2014). In our project, we have focused on the GGR taking advantage of the availability of the *Xpc* KO model in our laboratory. We assume that XPC deficiency leads to accumulation of DNA damages in HSC with age. Therefore, we studied young (age of 2.5 month \pm 0.4) and old mice (age of 16 month \pm 2.6) to unravel influence of aging in manifesting effects of XPC on maintenance of HSC.

A. XPC deficiency leads to increased body weight with aging

It has been described in previous reports that XPC deficiency is associated with increased incidence of internal tumors such lung cancer (He et al., 2013; Jin et al., 2014a), bladder cancer (Dai et al., 2014; Zhang et al., 2013), thyroid cancer (Santos et al., 2013) and digestive system cancer (Jiang et al., 2012). Studies also showed that *Xpc* KO model possess an extremely high incidence of lung tumors (Hollander et al., 2005b; Meira et al., 2001b; Melis et al., 2008b). The weight measurement of our mice has revealed a significant increase in weight of *Xpc* KO mice in comparison to wild type mice whether they are young or aged (Figure 49).

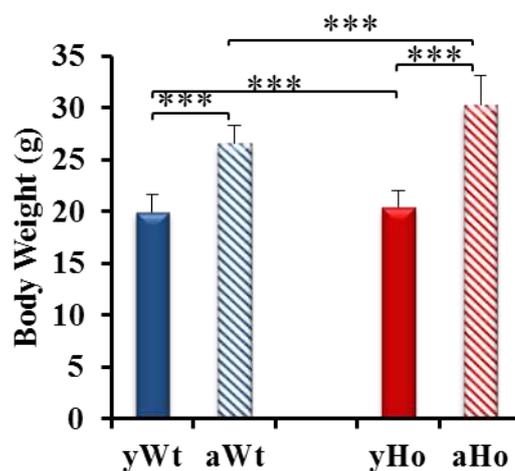


Figure 49. *Xpc*-knockout effect on total body weight. Graph representing the variation of the total body weight of different categories of *Xpc* mice. Results are shown as the mean \pm SEM. Young female mice (age of 2.5 month \pm 0.4) and old female

mice (age of 16 month \pm 2.6) were used for the experiments (yWT: n = 12; yHo: n = 14; aWT: n = 13; aHo: n = 13). ***p < 0.001. Symbols: y: young; a: aged; WT: wild type; Ho: homozygote.

B. *Xpc*-knockout effect on hematopoiesis

First, we investigated for modification of the hematopoietic organs in the *Xpc* KO model. We found increase in the weight of spleen in *Xpc* KO compared to WT mice, both young and aged. However, the ratio of spleen weight to total body weight is equivalent in all the groups of mice thereby indicating that the enlargement of spleen is certainly related to increase in weight mice (**Figure 50A**). In addition, the quantification of the number of BM (2 femurs, 2 tibias, and pelvis) cells shows that neither aging nor *Xpc* KO affect the BM cellularity (**Figure 50B**).

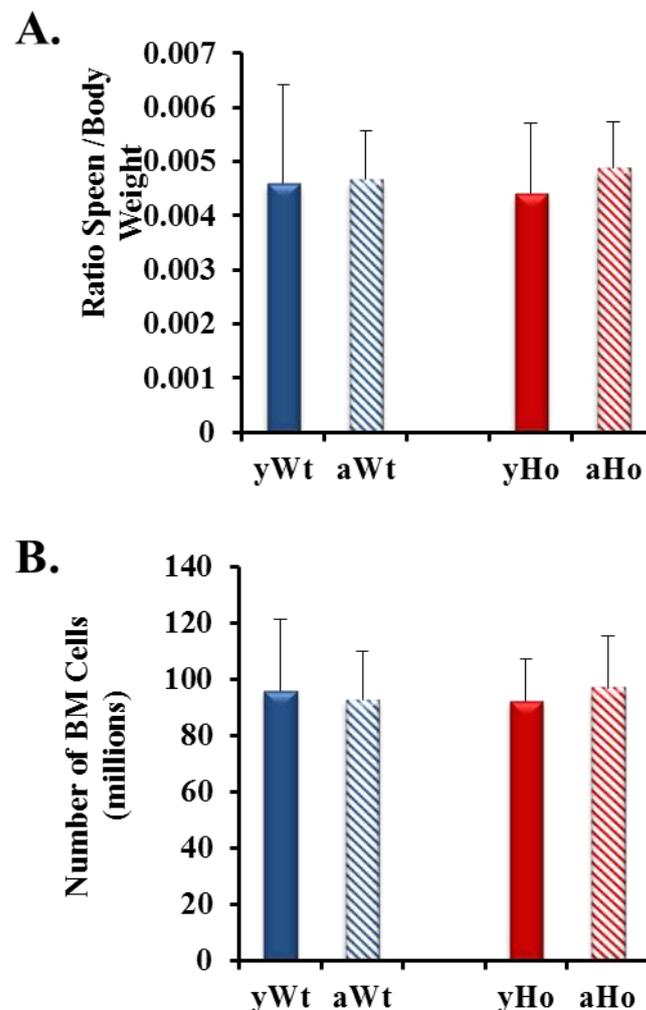


Figure 50. *Xpc*-knockout effect on spleen and BM cellularity. (A) Graph representing the variation of the ratio of spleen weight to total body weight of different categories of *Xpc* mice. (B) Graph representing the variation of the number of BM cells between the different categories of XPC. All results are represented as mean \pm SEM. Young female mice (age of 2.5

month \pm 0.4) and old female mice (age of 16 month \pm 2.6) were used for the experiments (yWT: n = 12; yHo: n = 14; aWT: n = 13; aHo: n = 13). *Symbols: y: young; a: aged; WT: wild type; Ho: homozygote.*

1. *Xpc*-knockout effect on mature BM and PB cells

Targeting the *Xpc* KO effect on mature hematopoietic cells in mice, the hematopoietic composition of mature cells of the PB and the BM was investigated. Analysis of hematopoietic composition in PB of *Xpc* KO has been determined by staining granulocytes (Gr-1), monocytes/macrophages (CD11b) and, B and T-lymphocytes (B220 and CD3 respectively) (**Figure 51A**). The data shows no significant difference in granulocyte and B-lymphocytes composition. A slight increase in monocytes/macrophages, concomitant to decrease T-lymphocytes in aged *Xpc* KO was observed compared to WT (**Figure 51B**).

Then, in order to assess the effect of *Xpc* KO on mature blood cells in BM, a study on the hematopoietic composition of BM of *Xpc* WT and KO mice that are either young or old was performed. The result of our analysis showed an increase in myeloid cells (CD11b) and decrease in lymphoid cells (B220) with aging in WT mice, however, there is no difference between WT and *Xpc* KO (**Figure 51C**).

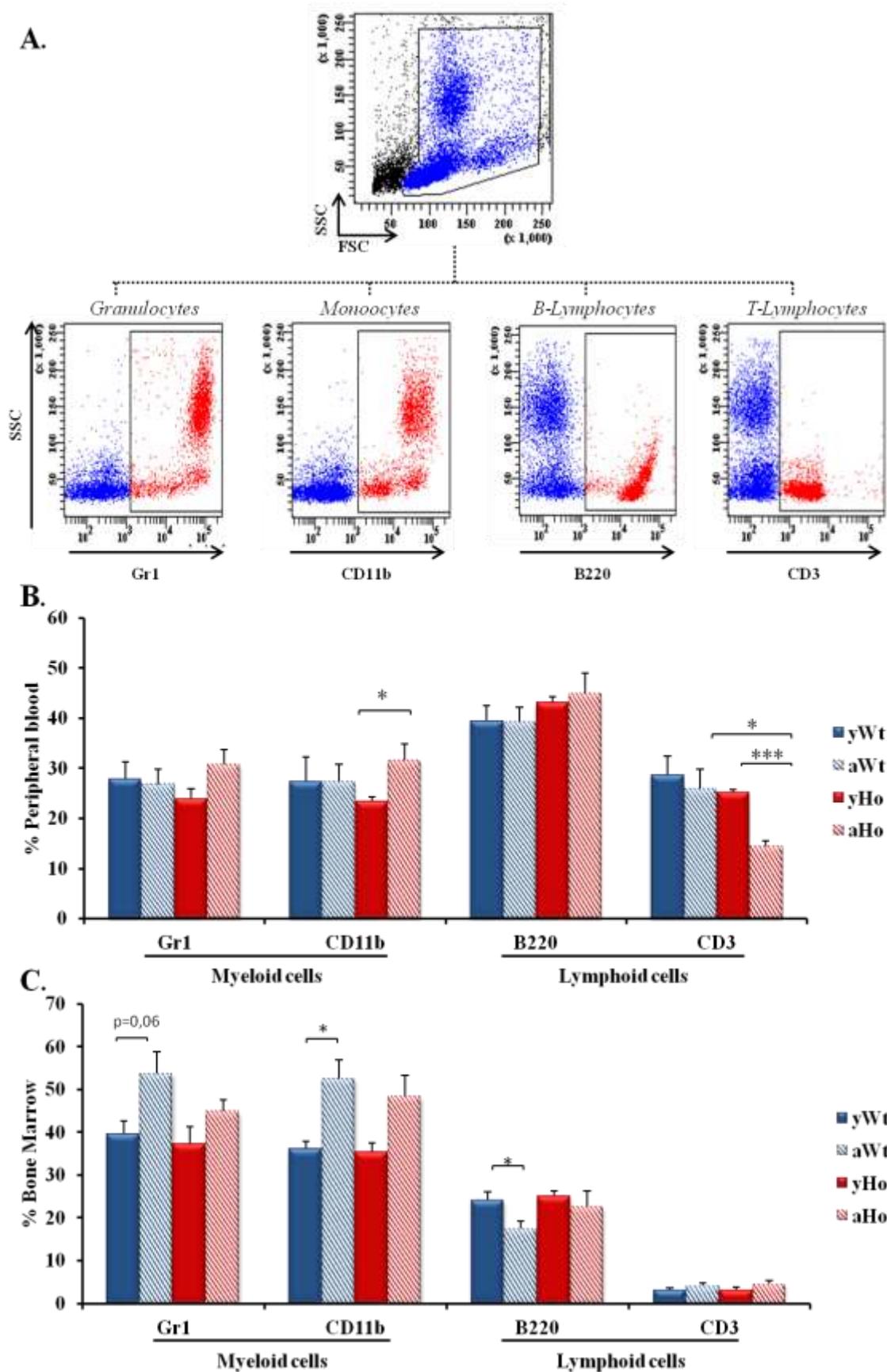


Figure 51. XPC deficiency leads to diminution of levels of T-Lymphocytes in PB of aged mice but has no effect of BM mature cells. (A) FACS image showing different hematopoietic cell populations obtained from lysed peripheral blood. Granulocytes, Monocytes, B- and T-Lymphocytes populations are identified by Gr1, CD11b, B220 and CD3 labeling of cells. **(B)** Histogram showing the variation in percentage of granulocytes (Gr1 marker), monocytes (CD11b marker), B- (B220

marker) and T- (CD3 marker) lymphocytes between the different mice categories (yWT: n = 12; yHo: n = 17; aWT: n = 13; aHo: n = 13). (C) Graph representing the amount of granulocytes (Gr1), monocytes (CD11b), B- (B220) and T- lymphocytes (CD3) present in bone marrow cells obtained from 2 tibias and 2 femurs of mice of different categories (n=4 each). All results are shown as mean \pm SEM. Young female mice (age of 2.5 month \pm 0.4) and old female mice (age of 16 month \pm 2.6) were used for the experiments. *p < 0.05, ***p < 0.001. Symbols: y: young; a: aged; WT: wild type; Ho: homozygote.

2. *Xpc*-knockout effect on stem BM cells

In the adult mouse, all multipotent cells are contained within the LSK fraction of cells in the BM (Ikuta and Weissman, 1992; Spangrude et al., 1988). However, the heterogeneous LSK cells can be further purified using two additional markers CD34 and flk2 into long-term reconstituting population known as LT-HSC (KLSflk2-CD34-), short-term reconstituting HSC known as ST-HSC (KLSflk2-CD34+), and multipotent progenitors known as MPP (KLSflk2+CD34+) (Rossi et al., 2005). Moreover, it has been reported that defect in DNA repair could lead to accumulation of mutations in the HSC which can lead to depletions of the BM with age (Niedernhofer, 2008b). Therefore, we decided to investigate the effect of the *Xpc* KO on the stem cell by analyzing its composition and whether there is an additional influence of aging (**Figure 52A**). First to evaluate the impact of aging on the steady-state frequencies of the stem cell subpopulations, we examined of the BM of young and aged mice and found that in the BM of aged mice the frequency of LT-HSC increased, the frequency of MPP cells decreased, and the frequency of ST-HSC cells did not appreciably change with age. Second, to investigate the effect of *Xpc* KO on the frequency of the stem cell subpopulations, we examined the BM of WT and *Xpc* KO mice. We did not find any impact of *Xpc* KO on the composition of stem cells in which the frequencies of LT-HSC, ST-HSC and MPP did not change in comparison with WT mice whether young or aged (**Figure 52B**).

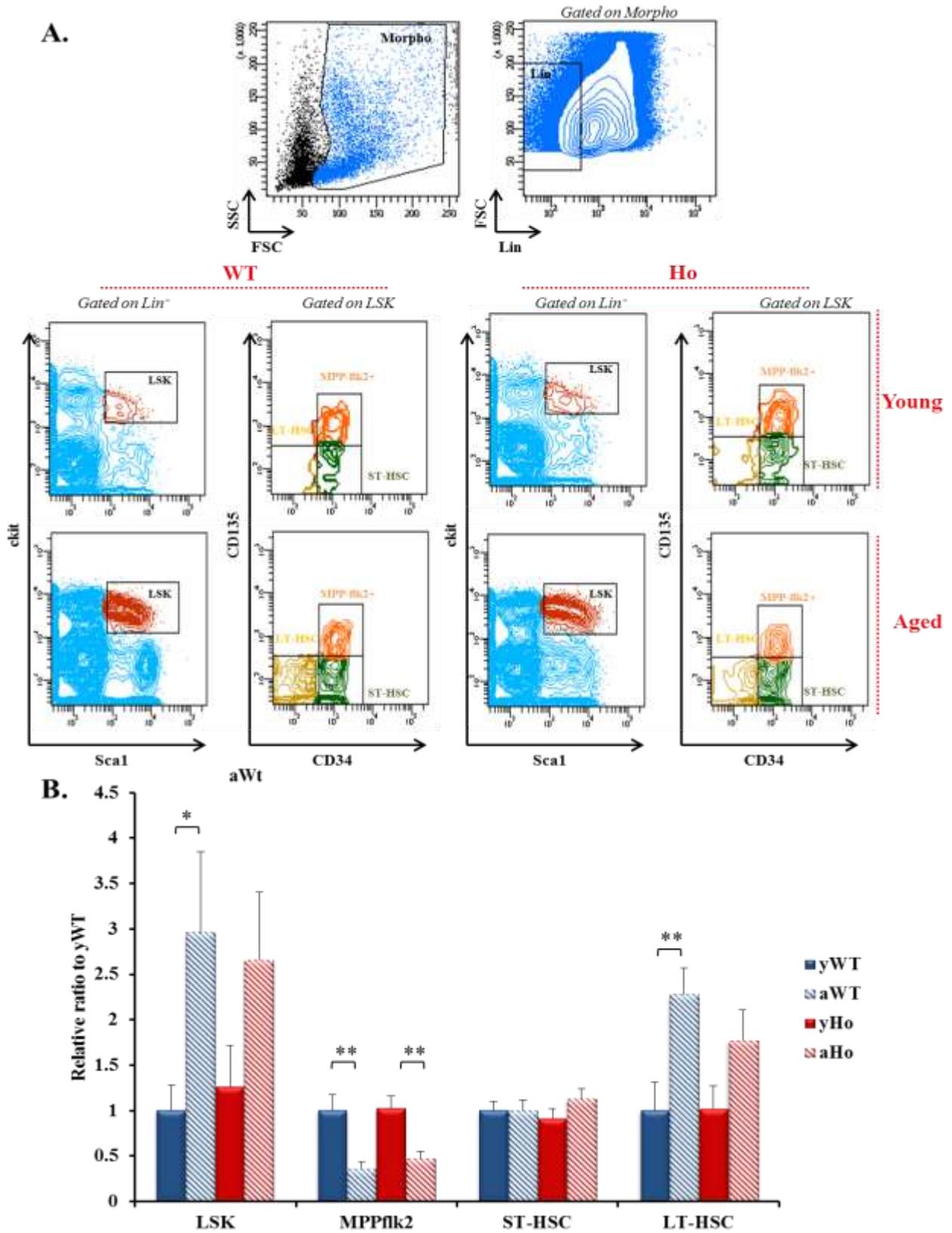


Figure 52. Use of LSK markers does not show an effect of XPC deficiency on stem cell composition in BM. (A) FACS gating procedure of BM cells to analyze stem cell composition. (B) Graph showing amount of LSK cells, LT-HSC, ST-HSC and MPP in BM obtained from different mice groups. Young female mice (age of 2.5 month ± 0.4) and old female mice (age of 16 month ± 2.6) were used for the experiments (yWT: n = 12; yHo: n = 14; aWT: n = 13; aHo: n = 13). Results are shown as the mean ± SEM. *p < 0.05, **p < 0.01. Symbols: Morpho: Morphology; Lin: Lineage; LSK: Lin⁻Sca1⁺ckit⁺; LT: Long-term; ST: Short-term; MPP: Multipotent progenitor.

In consequence of unexpected absence of effect of *Xpc* KO on the LSK cell populations using CD34 and flk2 markers, we have decided to further check the *Xpc* KO effect on more purified stem cell populations by using the SLAM markers CD48 and CD150 markers as a criterion for isolating HSC (Kiel et al., 2008). By this marking system, HSC are identified as LSKCD150⁺CD48⁻, MPP are identified as LSKCD150⁻CD48⁻, HPC-1 are identified as LSKCD150⁻CD48⁺ and HPC-2 are identified as LSKCD150⁺CD48⁺ (Oguro et al., 2013). Our data show an age-dependent increase in HSC frequency (known as SLAM population) and age-dependent decrease in HPC-1. Interestingly, our data show an increase in quantity of SLAM cells and decrease in quantity of MPP cells in aged *Xpc* KO mice in comparison to WT mice (**Figure 53**).

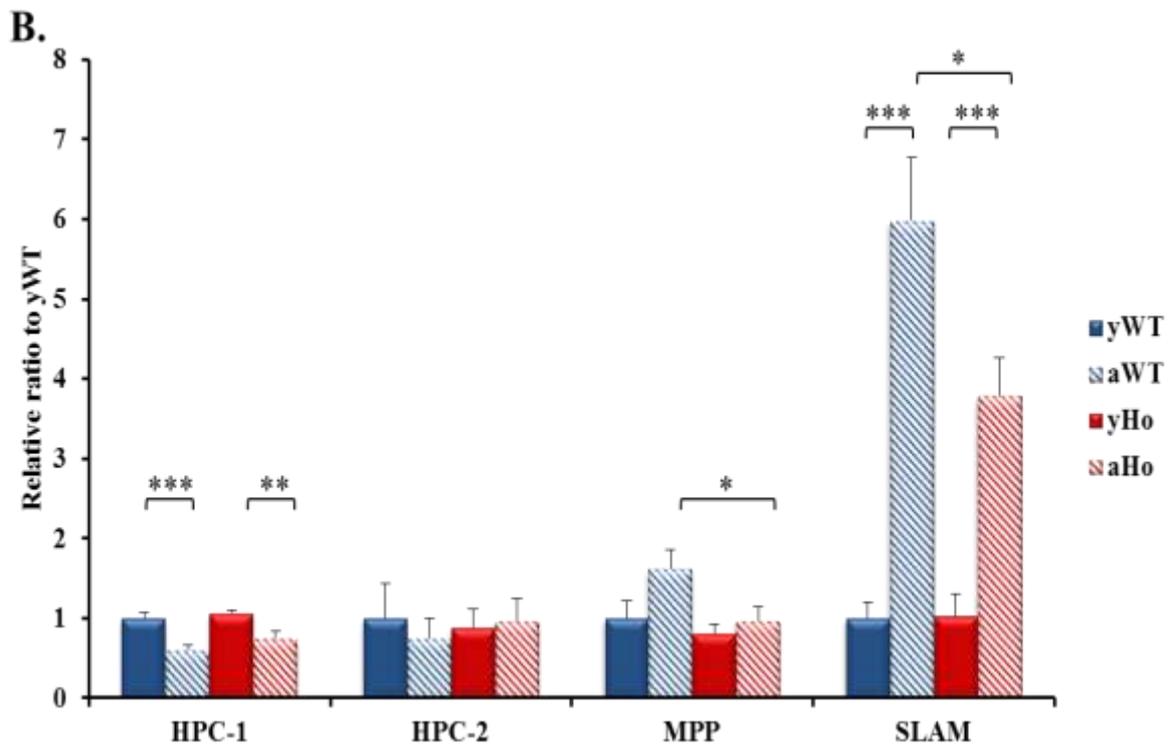
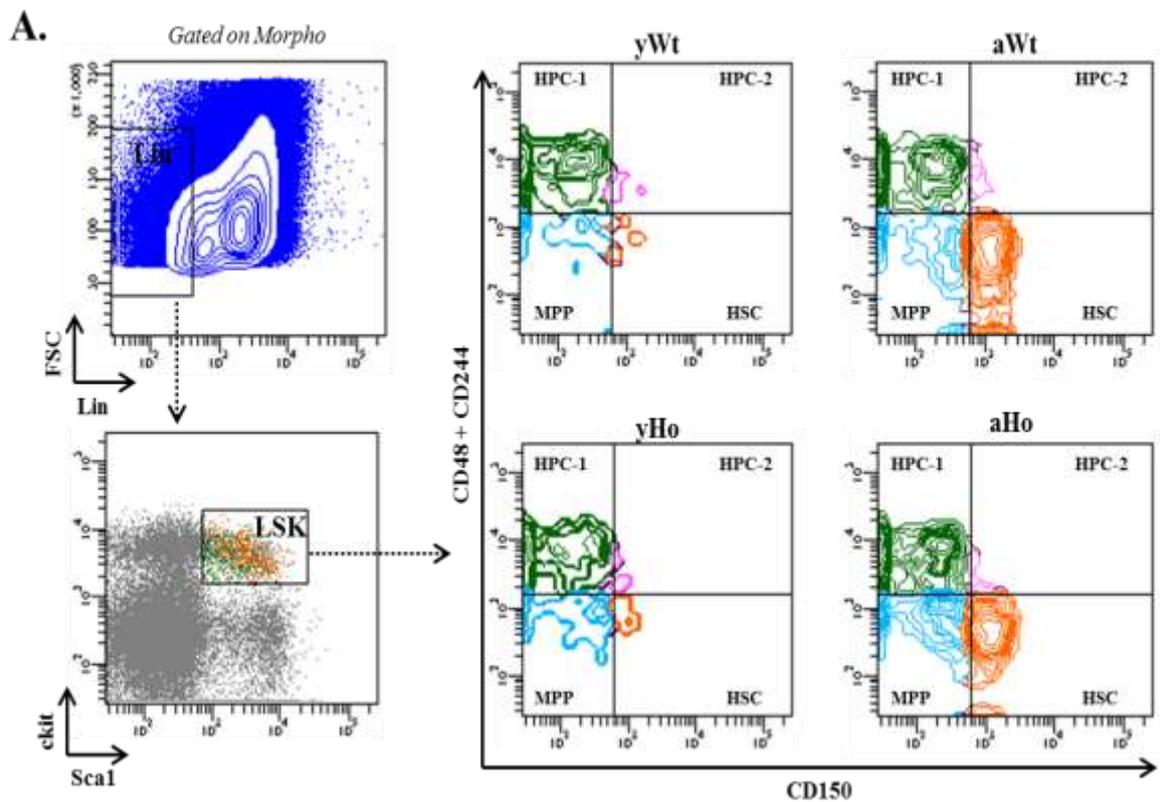


Figure 53. Use of LSK and SLAM markers reveal an effect for XPC deficiency on stem cell composition in BM. (A) FACS gating procedure of BM cells to analyze stem cell composition. **(B)** Graph showing amount of HPC-1, HPC-2, MPP and SLAM in BM obtained from different mice groups. Young female mice (age of 2.5 month \pm 0.4) and old female mice (age of 16 month \pm 2.6) were used for the experiments (yWT: n = 12; yHo: n = 14; aWT: n = 13; aHo: n = 13). Results are shown as the mean \pm SEM. * p < 0.05, ** p < 0.01, *** p < 0.001. Symbols: Morpho: Morphology; Lin: Lineage; HPC: Hematopoietic progenitor cell; LSK: Lin⁺Sca1⁺ckit⁺; MPP: Multipotent progenitor; SLAM: Signaling Lymphocyte Activation Molecules.

3. *Xpc*-knockout effect on BM progenitor cells

It is aforementioned that LT-HSC give rise to mature blood cells by differentiating through a succession of increasingly committed downstream progenitor cells (Kondo et al., 2003). The altered amount of SLAM cells that increase and MPP cells that decrease in aged *Xpc* KO mice compared to aged WT mice prompted us to examine the frequency of committed myeloid and lymphoid progenitors in aged animals (**Figure 54A**). Analysis of committed myeloid progenitors revealed that whereas the frequencies of CMPs were unaffected by age in both WT and *Xpc* KO mice, aged animals exhibited a significant increase in GMP cells in *Xpc* KO mice and significant decrease in MEP cells in both WT and *Xpc* KO model. Next, by examining the frequency of CLP cells in young/aged and WT/*Xpc* KO mice, we did not find any effect for aging or *Xpc* KO on CLP quantity (**Figure 54B**).

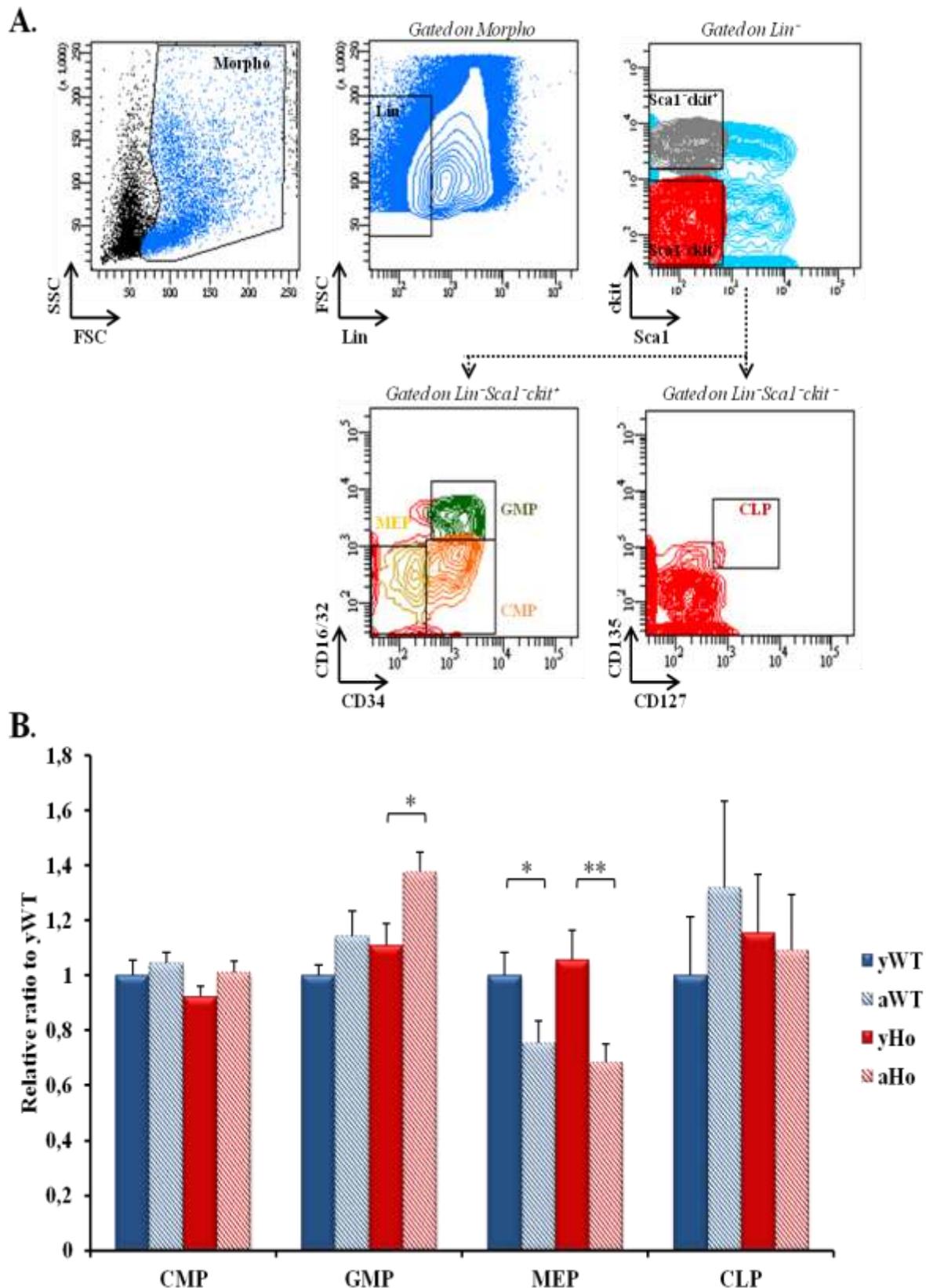


Figure 54. XPC deficiency does not affect progenitor cell composition in BM. (A) FACS gating procedure of BM cells to analyze myeloid and lymphoid progenitor cell composition. (B) Graph showing amount of CMP, GMP, MEP and CLP cells in BM obtained from different mice groups (12 yWT, 13 aWT, 14 yHo, 13 aHo). Results are shown as the mean ± SEM. *p < 0.05, **p < 0.01. Symbols: Morpho: Morphology; Lin: Lineage; CMP: Common myeloid progenitor; GMP: Granulocyte macrophage progenitor; MEP: Megakaryocyte erythroid progenitor; CLP: Common lymphoid progenitor.

Then we decided to study the impact of *Xpc* KO on functional capacity of BM-derived murine HSC to make progenitor. Our data showed an increase in number of CFC colonies with age in both WT and *Xpc* KO mice. However while there is no difference in the progenitor content between WT and *Xpc* KO mice we have observed that *Xpc* KO mice produce significant higher number of colonies than yWT mice (**Figure 55**).

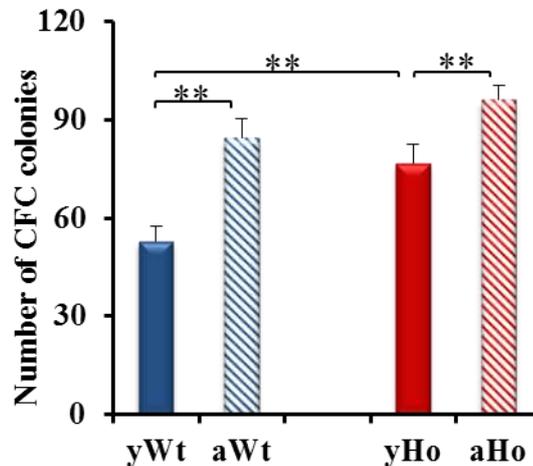


Figure 55. *Xpc* knockout effect on progenitor ability of young/aged mouse HSC. Graph representing variation in number of produced CFC colonies between the different categories of mice (n=7). Young (age of 2.5 month \pm 0.4) and old female mice (age of 16 month \pm 2.6) were used for the experiment. Results are shown as the mean \pm SEM. **p<0.01.

4. *Xpc*-knockout effect on ROS level in BM stem cells

To assess whether *Xpc* KO alter the oxidative metabolism in stem cells, ROS levels were evaluated in Lin^+ , Lin^- and LSK cells in the BM of the different mice groups (**Figure 56A**). Our results demonstrate an increase in the ROS level with age in Lin^- cells. However, we were not able to detect any change in ROS levels in Lin^+ , Lin^- and LSK cells in *Xpc* KO mice in comparison to WT mice (**Figure 56B**).

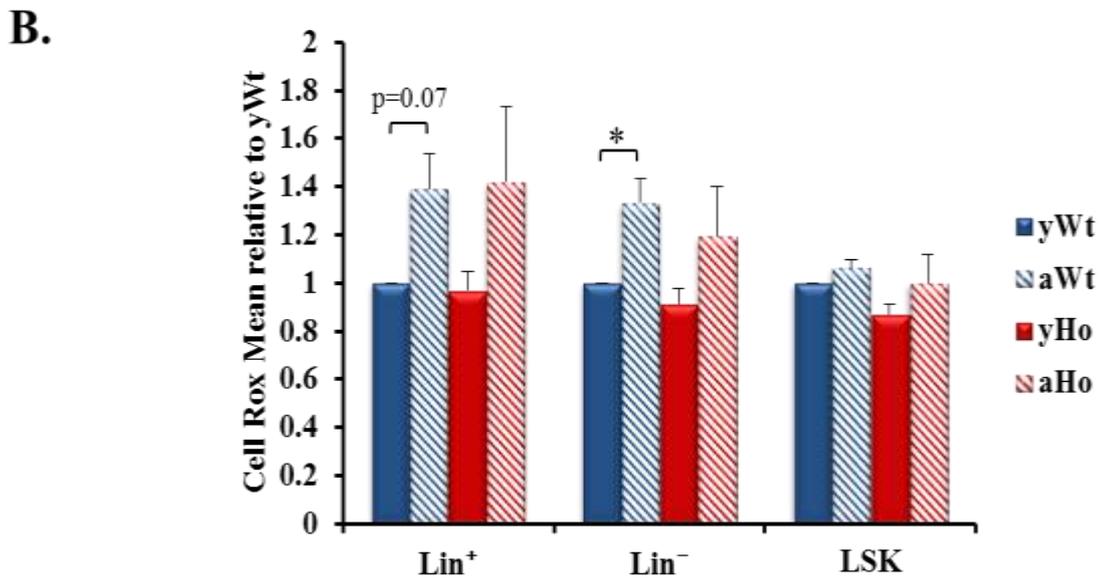
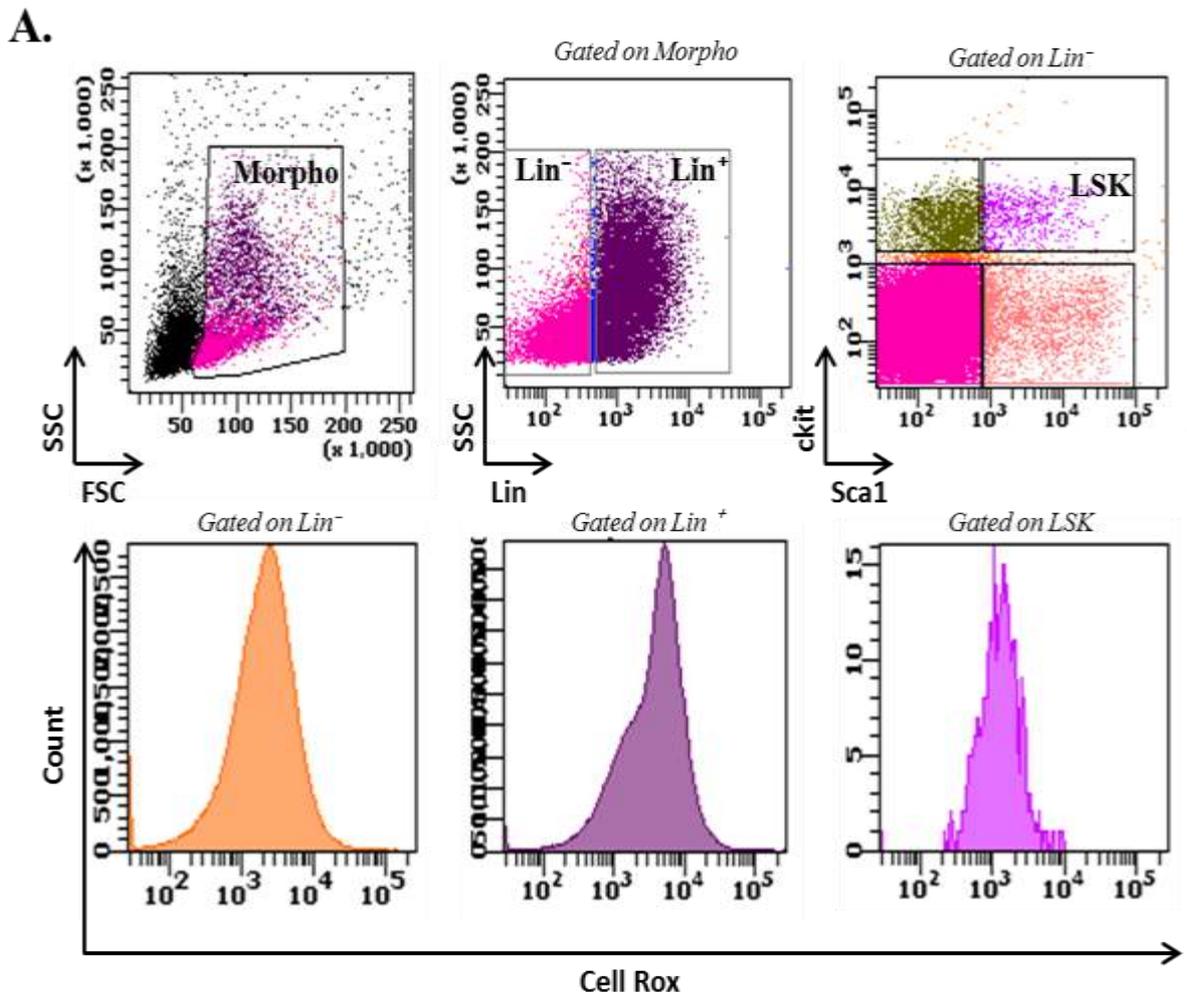


Figure 56. *Xpc*-knockout effect on ROS level in BM stem cells. (A) FACS image showing isolation of the Lineage-negative population (Lin⁻) followed by isolation of LSK cells (Lin⁻Sca1⁺c-kit⁺) using Sca-1 and c-kit labeling, then detection of ROS levels in Lin⁻, Lin⁺ and LSK cells. (B) Graph showing variation of relative ratio of CellROX mean in Lin⁻, Lin⁺ and LSK cells between different mice categories in comparison to yWT mice. Data represent mean of 4 experiments and the bars represent mean ± SEM. Young (age of 2.5 month ± 0.4) and old female mice (age of 16 month ± 2.6) were used for the experiment (yWT: n = 8; yHo: n = 10; aWT: n = 9; aHo: n = 10). Results are shown as the mean ± SEM. *p < 0.05.

C. Analysis of stress-induced hematopoiesis in *Xpc* KO mice

Previous studies have showed that *Xpc* KO mice had an altered response to carboplatin demonstrated by decreased survival, decreased progenitor ability and change in cell cycle (Fischer et al., 2009). *Xpc* KO mice has also showed differences in oxidative DNA damage sensitivity upon exposure if mice to paraquat (Melis et al., 2008b) and disturbed redox homeostasis (Rezvani et al., 2011a). Moreover, several lines of evidence has reported that XPC function not only in NER but also in BER (Melis et al., 2011, 2013a). Thus we assume that HSC will accumulate mutations rapidly under stress exposure and absence of XPC will be dramatic to maintenance of those cells. Therefore, we investigated the response to a genotoxic and replicative agent, 5-FU and the genotoxic gamma radiation.

Injection 5-FU in young mice induces similar cell death of WT and *Xpc* KO mice after two weeks suggesting that forcing stem cells to divide and to reconstitute full hematopoiesis did increase their apoptosis (**Figure 57A**).

X-ray irradiation induced DNA damages and increase ROS production in HSC (Wang et al., 2010; Yamaguchi and Kashiwakura, 2013). Therefore mice were subjected to increased (2 to 10 Gy) radiation. Survival was similar for WT and *Xpc* KO mice (**Figure 57B**). Some of the mice were sacrificed in order to enumerate the cellularity and perform progenitor-cell assay. Data showed not significant different neither in the cellularity nor in the frequency between WT and Ho mice (**Figure 57C-D**). Overall our data did not show an increased sensibility to replicative and genotoxic stresses when XPC is absent in hematopoietic cells.

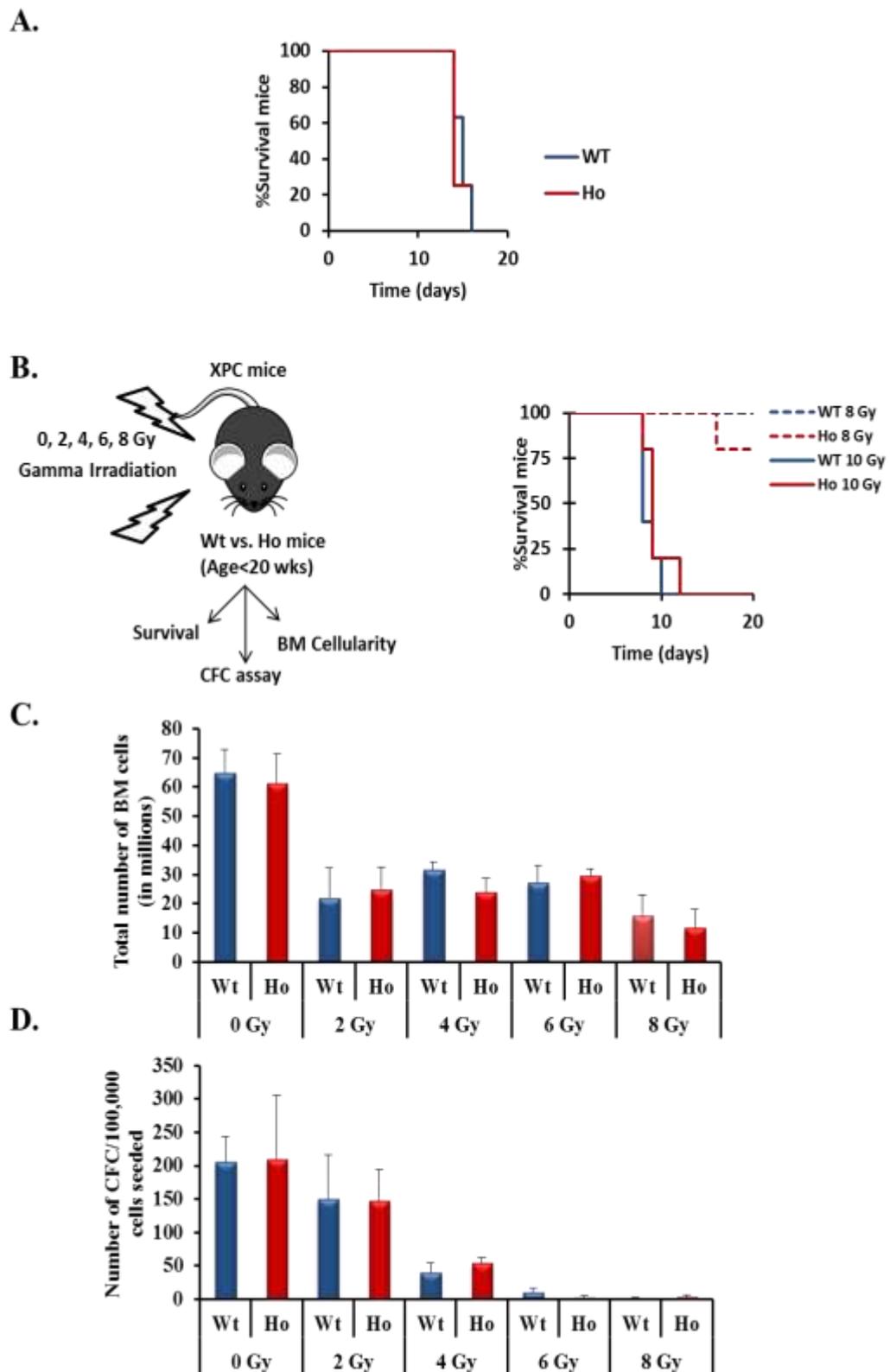


Figure 57. XPC deficiency does not alter response of mice to stress (5-FU, X-rays). (A) Survival curve of WT and Ho XPC mice subjected to weekly administration of the 5-Fluorouracil ($n = 5$ females and 5 males of each category). (B) On the left, a schematic diagram showing steps of the experiment performed. All mice were irradiated; some were used to check for survival of the mice, others were used to check for progenitor repopulation ability and bone marrow cellularity. Graph on right is Kaplan-Meier plot show survival of irradiated mice with 8 and 10 Gy ($n=5$ mice/ category/ dose). (C) Graph shows number of BM cells obtained from different mice categories irradiated with 0, 2, 4, 6 and 8 Gy ($n = 5$ mice/category/dose). (D) Graph shows number of CFC colonies produced per 100,000 seeded BM cells ($n = 5$ mice/category/dose).

Overall our data show that *Xpc* KO lead to increase in body weight, in the functional ability of stem cell to produce colonies in vitro, decreased T-lymphocyte production with aging in peripheral blood which is an indicator of thymic involution, increased SLAM quantities and decreased MPP with aging. Aging affected widely hematopoiesis where there was increase in quantity of myeloid and decrease in lymphoid cells in addition to increase in stem cell frequency (LSK, LT-HSC and SLAM), decrease in MPP quantities while ST-HSC quantities remained the same. We have also found that BM cellularity was not affected by aging or by XPC deficiency. Nevertheless, we did not find any change in ROS levels in BM stem cells between the different groups of mice. In addition, XPC was not involved in the response to stress induced by 5-FU administration or X-ray irradiation at the level of mouse survival, cellularity and progenitor ability.

Chapter 4

DISCUSSION AND PERSPECTIVES

HSC possess tight regulations of the balance between quiescence and proliferation, and between self-renewal and differentiation states. Integrity of HSC is threatened by abundant genotoxic, environmental and metabolic stresses along the life that could generate mutations and sometime trigger leukemogenesis necessitating the importance of DNA survey and repair mechanisms. Among the DNA repair pathways, NER is the most effective in removing bulky DNA lesions. NER importance in the long-term repopulation ability of HSC has been investigated using an *Xpd* KO model (Rossi et al., 2007a). However, XPD is involved in the two NER pathways, the GGR and TCR and, until now, the contribution of each branch in hematopoiesis has not been investigated. Since XPC is known as the initial factor in GGR, serving as a DNA-damage sensor, investigating it has seemed to be a good model for GGR separate from TCR (Friedberg et al., 2006). Therefore, in my thesis project, we have chosen to investigate GGR in maintenance of human and mouse HSC by studying the function of XPC in human UCB-derived CD34⁺ cells, using lentiviral silencing model, and in mouse BM-derived HSC, using *Xpc* KO model. Indeed, we speculated that, in absence of XPC, HSC will accumulate mutations triggering loss of HSPC and potentially will increase the risk of leukemogenesis when cell mechanisms bypass the mutations. Moreover, we hypothesized that severity of XPC deficiency on the maintenance of HSC might be enhanced with aging.

I. Human study

In this part of the project, we first investigated XPC expression in hematopoietic stem, mature and even in leukemic cells. Second we evaluated effect of *XPC* KD on UCB-derived CD34⁺ cells on multiple levels of hematopoietic hierarchy. The role of XPC in committed cells compartment was assessed by liquid culture of transduced CD34⁺ cells. The role of XPC in progenitor cells compartment was assessed by CFC assay. The role of XPC in stem cells compartment was assessed by LTR assay combining primary and secondary transplantations (**Table 9**).

Human study	XPC Effect		
	Assay	Observation	Human Hematopoiesis
Stem cell	LTR	Decline in Lin ⁻ CD34 ⁺ CD38 ⁻ , lymphoid, erythroid and myeloid lineage	Influence
Progenitor cell	CFC	No effect on progenitor ability of stem cells nor on the differentiation capacity since it does not affect the number of myeloid and erythroid colonies	No Influence
Mature cell	Liquid culture	No effect on proliferation and stability of XPC-silenced CD34 ⁺ cells	No Influence

Table 9. Summary of human study data. Symbols: LTR: Long-term repopulating assay; CFC: Colony-forming cell assay.

A. XPC expression correlates with the state of maturity of cell

Our results showed that XPC is highly expressed in CD34⁺ cells in comparison to its CD34⁻ counterpart at both the protein and transcript levels. In consistence, XPC protein expression decreased during liquid culture of CD34⁺ cells which was in parallel with the decrease of the percentage of CD34⁺ cells. Thus, we found that the decrease in XPC expression was correlated with differentiation of HSC. In agreement with our data, several studies have reported that the differentiation of cells is accompanied with downregulation of NER in general. For instance, GGR was found to be downregulated in terminally differentiated neurons (Nouspikel and Hanawalt, 2000a, 2002). Moreover, differentiation of AML cell lines was accompanied by a strong attenuation in the repair of cisplatin intrastrand GTG crosslinks (Hsu et al., 2007). In addition, NER was recently found to be downregulated in myeloid cells during differentiation. Microarray analysis of 100 AML patients showed that XPA binding protein 2 (XAB2) expression decreased during granulocyte differentiation and that the more differentiated cells were more susceptible to cisplatin than more immature myeloid leukemia cell lines (Aoki et al., 2014). Interestingly, NER downregulation was found to be associated with the cell cycle state of the cell. Hyka-Nouspikel et al. reported that NER is downregulated at the global genome level in quiescent human B-lymphocytes while maintaining proficient repair of constitutively expressed genes, which suggests the accumulation of DNA damage once B-lymphocytes are driven into proliferation. They have also found a molecular link between attenuation of NER during quiescence and cell mutagenesis in which NER attenuation in quiescent cells is associated with incomplete phosphorylation of the ubiquitin activating enzyme Ube1, a factor required for proficient NER (Hyka-Nouspikel et al., 2011).

Therefore, it is likely that GGR might be downregulated during differentiation of HSC into more mature cells which can explain our observed decrease in XPC expression or it is possible that XPC is more required in stem cells than in the downstream progenitors and mature cells.

Furthermore, we have found in mature peripheral blood cell fraction that XPC is mainly expressed in lymphocytes, monocytes but not in polynuclear cells. Since neutrophils which constitute the majority of polynuclear cells have high turnover rate, it is possible that the repair of DNA damage by GGR mechanism, which requires XPC as a damage sensor, is not dispensable to these cells. However, XPC prominent expression in lymphocytes suggests that XPC is important for these cells. For instance, Melis et al. has showed that XPC-deficiency is associated with increased mutation rates in lymphocytes (Melis et al., 2008c).

In addition, we have demonstrated a strong XPC expression in AML cancerous cell lines in which it presented different expression profiles from a cell line to another. In accordance with our data, Hsu et al. has showed that NER is downregulated in AML cell lines stimulated to differentiate by administration of phorbol esters in which repair of cisplatin was strongly attenuated (Hsu et al., 2007). Moreover, several studies have associated XPC deficiency with increased risk to leukemia (Guillem et al., 2010b; Strom et al., 2010) and altered susceptibility to the genotoxic effects of treatment.

B. XPC protein expression profile

Normally, the XPC molecular weight is described as the 125 kDa band as shown in keratinocytes (Rezvani et al., 2010). Surprisingly, the traditional full length (125 kDa) XPC protein was accompanied by the expression of two shorter proteins of ~100 and 110 kDa. The 125 kDa protein was upregulated in CD34⁺ cells in comparison to CD34⁻ cells oppositely to the 100 kDa protein band. In addition, during liquid culture of CD34⁺ cells, which induced cell commitment, we have traced the disappearance of the 125 kDa band and the upregulation of the other 2 bands. We were the first to report the existence of three protein bands for XPC in the hematopoietic cells and we attribute this finding to the use of a higher resolution in 8% SDS-polyacrylamide gel instead of the traditionally used 12% gel. Warrick et al. reported three protein bands of XPC (125, 110, 100 kDa) only when keratinocytes were forced to overexpress XPC (Warrick et al., 2012). These authors have reported that these short forms of XPC did not seem to act as dominant negative regulator of NER or to modify cell behavior. Warrick et al. hypothesized that the three bands maybe mRNA splicing isoforms. Indeed, two in-frame AUG codons exist in the XPC coding sequence downstream the bona fide initiation

codon and, theoretically, translation initiation from these codons could generate proteins of 823 (~110 kDa) and 747 (~100 kDa) amino acids respectively. Furthermore, Legerski et al. reported a cloned human *XPC* complementary DNA that lacks the 117 first amino acids, presumably corresponding to the 110 kDa band. Interestingly, expression of this protein in immortalized *XPC* cells restored normal UDS levels and UV survival properties (Legerski and Peterson, 1992). Nevertheless, until now the function of the 100 kDa band remains ambiguous in which it has not been investigated previously.

The different protein forms of *XPC* in hematopoietic cells opened new speculations on the origin of three bands which might be explained by certainly transcriptional or/and post-transcriptional modifications or/and degradation. A link between the modifications and the functional activity or resistance to various cell stresses could be also hypothesized. We showed that the three bands are not splicing variants suggesting most probably post-translational modification. One classical modification is phosphorylation. For instance, *XPC* has been reported to be phosphorylated by ATM and ATR pathways under stress condition (Matsuoka et al., 2007; Wu et al., 2006). WIP-1, a serine/threonine wild-type p53-induced phosphatase 1, has been reported to negatively regulate NER by dephosphorylating *XPC* and *XPA* leading to its inactivation (Nguyen et al., 2010). Other post-translational modifications were reported in literature for *XPC*. *XPC* is ubiquitinated during the NER process by DDB2, which cause an increase in the affinity of *XPC* for DNA (Sugasawa, 2006b; Sugawara et al., 2005; Wang et al., 2007). Another example is that *XPC* can be sumoylated upon UV irradiation by SUMO-1 which is believed to stabilize *XPC* (Wang et al., 2005). Nevertheless, we argue that it is not possible that *XPC* might be ubiquitinated or sumoylated since addition of ubiquitin or SUMO moiety will result in much bigger bands. Indeed, Wang et al. has documented that ubiquitination or sumoylation of *XPC* will yield protein bands of much higher molecular weight (145-220 kDa) (Wang et al., 2005). It is also possible that the *XPC* bands might be due to degradation as it has been previously noted that *XPC* degradation results by direct interaction with the proteasome (Wang et al., 2007).

C. Function of *XPC* in human hematopoiesis

The function of *XPC* has been investigated by evaluating the effect of its KD in CD34⁺ cells during liquid culture *in vitro*, and during immune-deficient NSG mice transplantation *in vivo*.

Following the validation of the efficiency of sh*XPC*-GFP lentivirus in silencing *XPC* expression in CD34⁺ cells, the follow-up of the amount of sh*XPC*-transduced cells by their emitted GFP signal during liquid culture indicated a prevalent stability. The proliferation rate

and the percentage of the CD34⁺ cells were also not affected by *XPC* KD. Moreover, *XPC* KD had null effect on the clonogenic capacity of CD34⁺ progenitors. Likewise, *XPC* KD did not affect the number of myeloid and erythroid colonies generated pointing an absence for *XPC* effect on differentiation capacity. However, we strongly postulate that if we have exposed our cultured cells to stress factor, such as irradiation (UV light, gamma rays) or chemical administration (cisplatin, carboplatin, arsenic, benzene), we could have found a modified stress response in the *XPC* KD cells *in vitro*. In support to our speculation, *XPC* transcript level was reported to increase when HSC were exposed to benzene (Faiola et al., 2004). Under exposure to arsenic trioxide, *XPC* silencing was accompanied with an increased susceptibility of human glioma cells to death (Liu et al., 2010). Under cisplatin treatment, *XPC* silencing affected the transcription levels of cell cycle and cell-proliferation related genes and modulated the cisplatin-treatment mediated DNA repair process (Wang et al., 2004).

After that, *XPC* KD effect on stem cells has been investigated by follow-up of the engraftment and lineage reconstitution ability of the *XPC* KD cells in NSG mice primary and secondary recipients. Indeed, challenging the functional capacity of stem cells by transplantation into immunodeficient NSG mice provides a powerful assay system to characterize the HSC compartment (Bonnet, 2002). And since the production of different cell types in xenografts is temporally restricted in which not all lineages may be readily assayed at any given time point, we decided to follow a careful kinetic assessment of the engraftment. In primary mice transplants, our data showed a progressive depletion in human engrafted cells silenced for *XPC* expression that reaches significance after 12 weeks and a more dramatic drop was observed after 18 weeks. Further analysis of *XPC* effect on the lineage reconstitution has revealed that *XPC* KD has lead to perturbation in the erythroid, myeloid, and lymphoid lineages after 18 weeks of transplant. Moreover, after 18 weeks of transplant, an equivalent diminution in the frequency of the immature stem cell subpopulations was traced in *XPC* KD mice. The prominent effect of *XPC* on stem cell maintenance was further confirmed by secondary transplantation which witnessed a major depletion in the quantity of human engrafted cells in the *XPC* KD mice. Thus, our data show modulation of *XPC* in hematopoietic cells and demonstrate that *XPC* participates to stem cell maintenance whereas this factor is not required for committed cell proliferation as initially suggested by the low expression in CD34⁻ cells.

Our data majorly show that *XPC* is important in maintenance of HSC which is in agreement with several other reports. For instance, *XPC* has been found to be implicated in embryonic stem cell maintenance through the direct interaction of *XPC*-*RAD23B*-*CETN2* complex with

Oct4 and Sox2. This interaction has been shown to be a requisite for embryonic stem cell self-renewal and efficient somatic cell reprogramming (Fong et al., 2011). We suggest that XPC effect on maintenance of CD34⁺ cells *in vivo* is due to role of XPC outside of NER. Le May et al. has demonstrated that, under absence of exogenous genotoxic stress, XPC protein is indispensable for the assembly of NER factors to the transcription site which permits the achievement of an optimal DNA demethylation and histone posttranslational modifications thereby allowing an efficient RNA synthesis (Le May et al., 2010a). Thus it is possible that the novel discovered role of XPC in transcription could be behind its important effect on the HSC maintenance. It is also possible that the role of XPC in BER in which it participates in the repair of oxidative DNA damage could explain our observed result (Melis et al., 2013a).

D. XPC implication in oxidative stress

Previous studies have pointed to a role for XPC in repair of oxidative DNA damage. XPC has been shown to interact with several glycosylases such as 3-methyladenine DNA glycosylase (Miao et al., 2000), thymine DNA glycosylase (TDG) (Shimizu et al., 2003b), selective monofunctional uracil DNA glycosylase (SMUG1) (Shimizu et al., 2003b), and 8-oxoguanine DNA glycosylase (OGG1) (D'Errico et al., 2006). Moreover, XPC KD cells showed higher ROS levels compared to control fibroblasts (Fréchet et al., 2008b). Rezvani et al. demonstrated that XPC defect generates oxidative stress in the cell as measured by elevation of ROS levels (Rezvani et al., 2011a, 2011b). A recent study by Menoni et al. has showed that XPC had a role in protection of cells against oxidative DNA damage where it was rapidly recruited to oxidative DNA lesions in living cells (Menoni et al., 2012). Therefore, we have chosen to assess the ROS levels in stem/progenitor cell from NSG bone marrow. However, our study did not demonstrate increase in ROS in XPC KD stem/progenitor cell compared to normal cells. In support to our data, HSC are known to cycle very infrequently and primarily reside in the relatively inactive metabolic phase G0 of the cell cycle (Bradford et al., 1997) which exposes them to lower levels of damage-inducing metabolic side products and ROS than more metabolically active differentiated cell types (Bryder et al., 2006). On the other hand, stem cells have been reported to express high levels of numerous ABC/MDR transporter genes (Zhou et al., 2001) whose products are known to have physiological roles in cytoprotection. Piccoli et al. pointed out the fact that HSC has to be pre-treated with cyclosporine A, an inhibitor of multi-drug resistant pump (MDR1) that allows the substantial trapping of the probe inside the cell. Hence, not using cyclosporine might allow the escape of ROS detection (Piccoli et al., 2005, 2007). However, it can be also argued that CellROX

probe measure the total ROS level which could be constant due to balance effect in the oxidative metabolism. Thus instead we should have measured separately the cytosolic ROS and the mitochondrial ROS using more specific probes.

II. Mouse study

Several publications have handled hematopoietic studies on NER-deficient models, such as *Xpa* KO model mice, which show a significant decrease in CFU-GM colonies only in aged mice (57 wks) but no difference in PB value or BM count (Prasher et al., 2005). The study of the hematopoiesis in *Xpd* KO model has showed no effect on the stem cell reserve with age but showed severe effect on stem cell functional capacity under conditions of stress leading to the loss of reconstitution and proliferative potential, diminished self-renewal, increased apoptosis and, ultimately, functional exhaustion (Rossi et al., 2007a). Furthermore, analysis of hematopoiesis in *Xpf* KO mice model showed that it possessed multilineage cytopenia, fatty replacement of BM, significant reduction in proliferative reserve of hematopoietic progenitors and stress erythropoiesis, effect manifested with aging (Prasher et al., 2005).

In our study, we have used the *Xpc* KO model which provides a valuable tool for modeling the human XPC disease in mice and allows us to investigate the effect of XPC on the murine hematopoiesis and the maintenance of the mouse HSC. In the literature, several studies have associated XPC defect with defects in the bone marrow hematopoiesis. For instance, a case-report has been published previously on an XPC patient with myelosuppression (Salob et al., 1992). Moreover, a prior study exist on the bone marrow of *Xpc* KO mice subjected to carboplatin (Fischer et al., 2009). However, Fischer et al. used an *Xpc* KO mice model (Sands et al., 1995) which is different from the model that we have used (Cheo et al., 1997). Noteworthy, several clues in the literature led us to suggest that impact of *Xpc* KO is enhanced with aging. For instance, Rube et al. found that DNA damage accumulate in human HSC with aging (Rübe et al., 2011). Rossi et al. has showed that an age-dependent spontaneous or endogenous DNA damage accumulation was behind the loss of HSC function in DNA repair-deficient mice that were not exposed to exogenous genotoxic stress (Niedernhofer, 2008a; Rossi et al., 2007a). Thus, we have also integrated the aging factor in our study by investigating young and aged *Xpc* KO mice and compare them to their wild-type littermates.

In our study, we held an immense investigation on the hematopoietic composition in both peripheral blood and bone marrow of young and aged *Xpc* KO mice. We have analyzed the

frequencies of stem cell populations, progenitor cell populations and mature cell populations by flow cytometry. Moreover, we assessed the progenitor ability of young and aged mice BM-derived HSC by progenitor cell assay in normal conditions or under stress by irradiation (Table 10).

XPC model study		Aging Effect		XPC effect		
		Young/Old		WT/Ho		
		WT	Ho	Young	Old	
Total Body Weight		↑	↑	↑	↑	
BM cellularity						
Colony-forming ability		↑	↑	↑		
Peripheral blood	Granulocytes					
	Monocytes		↑			
	B-lymphocytes					
	T-lymphocytes		↓		↓	
Bone marrow	Mature cells	Granulocytes				
		Monocytes	↑			
		B-lymphocytes	↓			
		T-lymphocytes				
	Stem cells	LSK	↑			
		SLAM	↑	↑		↑
		LT-HSC	↑			
		ST-HSC				
		MPP (LSKCD34 ⁺ flk2 ⁺)	↓	↓		
		MPP (LSKCD150 ⁻ CD48 ⁻)				↓
		HPC-1	↓	↓		
		HPC-2				
		CMP				
		GMP		↑		
	MEP	↓	↓			
	CLP					
ROS	Lin ⁻	↑				
Murine Hematopoiesis		Major Influence		Minor influence		

Table 10. Summary of mouse study data. Symbols: WT: Wild type; Ho: Homozygote; HPC: Hematopoietic progenitor cell; ROS: Reactive oxygen species; NOX: NADPH oxidase activity.

A. XPC do not impact murine hematopoiesis

Our data show significant increase in weight in aged *Xpc* KO mice compared to aged WT. We speculate that this increase in weight is due to metabolic switch. In support to our assumption, Rezvani et al. has previously reported that XPC deficient cells modify their metabolism from oxidative phosphorylation to glycolysis. However, the increase in weight could be due possibly to acquisition of tumors.–For instance, it has been reported that with age *Xpc* KO mice possess lung cancer (Hollander et al., 2005a) and increased predisposition to spontaneous internal cancers (Kraemer et al., 1987; Meira et al., 2001a; Melis et al., 2008c).

Our data did not show difference in the BM cellularity with aging which is in consistence with findings of Fedoročko et al. in which no change in BM cellularity was reported in old female ICR mice (Fedoročko et al., 2000). Furthermore, our data showed no influence of XPC deficiency on the BM cellularity. In accordance with our finding, the investigation of *Xpd* KO mice revealed that NER-deficiency did not affect the stem cell numbers in the BM but its function (Rossi et al., 2007a). However, our result is not in agreement with results published by Fischer et al. on *Xpc* KO mice in which he demonstrated that XPC deficiency leads to decrease in BM cellularity.

In addition, under normal conditions, PB analysis showed a significant increase in frequency of monocytes and decrease in frequency of T-lymphocytes with aging in *Xpc* KO mice but weirdly not in wild type mice. In support to our data, Melis et al. has reported that *Xpc* KO mice had a significant increase in acidophilic macrophage pneumonia (Melis et al., 2008a). In the literature, numerous reports have illustrated that during aging there is lineage dysregulation represented by decrease in lymphoid cell amounts and increase in myeloid cell amounts (Kim et al., 2008; Rossi et al., 2005). However, our aged mice were only 16 month old while most studies on aging used mice older than 20 months. We can attribute the discrepancy between our results and previous ones to the various ages of the mice. In our experiments, we used young mice of age 2.5 month \pm 0.4 and old mice of age 16 month \pm 2.6. Noteworthy, it has been reported that *Xpc* KO mice develop spontaneous lung cancers at one year of age (Hollander et al., 2005a) which prompted us to use mice as young as possible for the current study. Our choice of age was similar to that of Rossi et al. who examined the impact of DNA repair deficiency on hematopoietic composition in 2.2 month old young and 14.5 month old aged *Xpd* KO mice (Rossi et al., 2007a). Though, in the published work about stem cell aging, Rossi et al. has used older mice that are aged up to 23 month (Rossi et al., 2005). Besides, recently Beerman et al. has reported the use of 3-4 month old young mice and 24-26 month old aged mice (Beerman et al., 2014). Additionally, Quéré et al. used 4 month

old young mice and 20 month old aged mice in an attempt to study aging phenomenon (Quére et al., 2014).

Markedly, the inspection of the hematopoietic composition of the PB has showed no effect of *Xpc* KO on amounts of granulocytes, monocytes and B-Lymphocytes but has revealed a decreased amount of T-Lymphocytes in aged *Xpc* KO mice in comparison to aged WT mice. We suggest that *Xpc* KO lead to depletion of T-lymphocytes in PB of aged mice due to accumulation of oxidative stress or thymic involution. Additionally, XPC seems to play a role in lymphocytes integrity. In support of our assumption, studies on lymphocytes from *Xpc* KO mice noted accumulation of spontaneous lesions, which result from oxidative processes, suggesting a causative role for ROS (Wijnhoven et al., 2000). As well, using yeast hybridization system to unravel XPC protein interactome, XPC was shown to interact with T cell gamma variable 4 (TRGV4) protein which is a T cell receptor involved in the immune response and function in signal transduction. XPC has also been shown to interact with DHX32 protein, a member of the DEAD box proteins, known to function in transcription regulation and to be implicated in lymphoid maturation and immune response (Lubin et al., 2014). Remarkably, lymphoid function was also lost in *Atm* KO mice, known to be defective in HR pathway and thereby in repair of DSB breaks, along with progressive BM failure resulting from a defect in HSC function, with a decreased stem cell number, loss of repopulation capacity, and increased apoptosis that was associated with elevated reactive oxygen species which was corrected by treatment with anti-oxidative agents (Ito et al., 2004b).

The analysis of mature cells in BM has showed an increase in monocytes and decrease in B-lymphocytes with aging in WT mice. Our data are consistent with numerous publications which reveal that the frequency of myeloid cells increase with aging while the frequency of lymphoid cells decrease with aging. In addition, it has been showed that the altered lineage potential of LT-HSC with aging is underwritten by age-dependent changes in gene expression at the stem cell level. Rossi et al. has demonstrated that LT-HSC aging is accompanied by systematic down-regulation of genes mediating lymphoid specification and function and up-regulation of genes mediating myeloid specification and function (Rossi et al., 2005). However, no differences in the frequency of mature BM cells were detected between *Xpc* KO and WT mice.

The analysis of the progenitor cell composition in the BM has shown that aging was accompanied by an increase in GMP frequency and no change in CMP frequency which is

consistent with the literature. Unexpectedly, aging provokes a decrease in MEP frequency and no modulation in CLP frequency. However, previous publications have showed that aging phenomenon was accompanied with decrease in CLP frequency and no change in MEP (Rossi et al., 2005). Indeed, Rossi reported that CLP decrease with aging after 22 month and this decrease is significant in comparison to 12 month-old mice. Hence, we suggest that the reason behind the discrepancy between our results and the literature is that our mice are not aged enough as mentioned previously. In support to this assumption, CLP cells have a lower proliferative index than granulocyte-macrophage progenitors which gives rise to mature granulocytes that turn over very rapidly (Passegué et al., 2005), presumably resulting from the fact that the progeny of the CLP are more long-lived B and T cells, which themselves are capable of extensive proliferation during maturation. Furthermore, we did not find any influence for *Xpc* KO on the amount of the progenitor cells whether in young mice or aged mice.

Despite the finding that *Xpc* KO did not alter the frequency of progenitor cells in the BM, by the use of CFC assay we have found that the number of colonies produced by the BM cells were higher in *Xpc* KO mice compared to WT mice signifying an increased progenitor ability of *Xpc* KO cells. In contrary, Fischer et al. reported that the colony-forming ability of XPC-deficient BM cells was decreased 3-fold compared to wild type (Fischer et al., 2009). However, Fischer used another *Xpc* KO model from Sands et al. as we have already mentioned and most importantly he based his result on data from only three mice of each genotype which is not trustworthy. We have also demonstrated that number of colonies generated by BM cells of WT and *Xpc* KO mice increase with age. In support to our data, Fedoročko et al. also demonstrated an age-dependent increase in the number of granulocyte-macrophage colony-forming cells (GM-CFC) and erythroid burst-forming units (BFU-E) in the BM of two-year-old female ICM mice (Fedoročko et al., 2000). Recently, Beerman et al. demonstrated that XPC in young mice is more expressed at the transcriptional level in MPP, CLP and BLP in comparison to HSC but less expressed in MEP and does not differ in CMP and GMP while in old mice XPC is more expressed in GMP, CLP and BLP but less expressed in MEP in comparison to HSC (Beerman et al., 2014). In addition, attenuation of DNA repair in HSC in comparison to downstream progenitor populations has been demonstrated. Thus, the higher expression of XPC in progenitors than in the HSC itself indicate that, most probably in murine hematopoiesis, XPC exerts an effect at the progenitor cell level.

B. XPC impact on HSC populations

At the stem cell level, we evaluated the hematopoietic composition of the bone marrow in the four groups of mice. Using LSK stem cell staining system, we have observed an increase in frequency of LSK and LT-HSC, decrease in frequency of MPP and no change in frequency of ST-HSC with aging which is consistent with other reports of aging (Quéré et al., 2014; Rossi et al., 2005). However, we did not find an effect for *Xpc* KO on the HSPC composition. Using SLAM stem cell marking system, we have noticed a significant increase in frequency of SLAM cells with aging and decrease in HPC-1 progenitor cells. We have found an effect for *Xpc* KO on the stem cell composition with aging manifested by increased SLAM quantity and decreased MPP quantity. As an explanation to our results, it has been reported that aging is manifested by accumulation of DNA damage upon NER deficiency (Niedernhofer, 2008a; Rossi et al., 2007b). In our *Xpc* KO model, we propose that the accumulated DNA damage with aging mediate a change in the cell intrinsic property of the SLAM HSC provoking an increased self renewal and decreased differentiation potency which could explain the observed increase in frequency of SLAM counterbalanced by decrease in frequency of MPP.

Finally, we were expecting much greater effect for *Xpc* KO on murine hematopoiesis. However, recently, Beerman et al. has showed that despite HSC accumulate DNA damage during aging, the DNA repair pathways including NER are attenuated in quiescent HSC but become activated upon entry into cell cycle. So, when HSC are stimulated to enter into cycling state, genes belonging to DDR and DNA repair pathways are upregulated and the damages are repaired. Moreover, according to Beerman et al., unexpectedly, and in contrast to most other DNA repair genes, when young and old HSC were stimulated into cycling phase, XPC expression decreased starting from 3 hr post stimulation and this decrease continued till 24 hours post stimulation. Therefore XPC may not be a major player in HSC maintenance and it is very possible that overlapping pathways exist in the mice that might compensate its loss in the cell.

C. XPC impact on oxidative stress in HSC

Several lines of evidence have suggested that XPC-deficiency in BM could create oxidative stress. For instance, Fischer et al. has found that *Xpc* KO mice treated or untreated with carboplatin showed a defective ubiquitinylation of CUL4A and CDT1 cell cycle protein which was associated with a role of XPC in signaling of oxidative base damage (Arias and

Walter, 2007; Hu et al., 2004; Sugasawa et al., 2005). Additionally, our team have previously found that *XPC* KD leads to activation to Akt1 and NOX1, which then trigger elevation of ROS levels, mtDNA deletions and neoplastic transformation to squamous cell carcinoma (Rezvani et al., 2011a, 2011b). Our data of ROS measurement in BM-derived HSC showed only a significant increase of ROS in aged Lin⁻ cells while no change of ROS was detected in *Xpc* KO mice. We suggest that the absence of *Xpc* KO on ROS levels is due to the fact that adult HSC reside in hypoxic niches in the BM cavity and are mostly kept in a quiescent phase thereby leading to fewer exposure to DNA damage associated with ROS production, cellular respiration and cell division contributing to their overall maintenance and protection of their genomic integrity (Orford and Scadden, 2008; Rossi et al., 2007a).

D. XPC impact on HSC stress response

Numerous studies have showed an effect of XPC on stress-mediated response. Fischer et al. studied the effect of *Xpc* KO in response to carboplatin. His study demonstrated that *Xpc* KO mice had decreased survival capacity upon carboplatin treatment and the BM of *Xpc* KO mice exhibited 10-fold greater sensitivity to carboplatin compared to WT BM (Fischer et al., 2009). Nonetheless, our data did not show an effect for *Xpc* KO on response of HSC to irradiation (>2 Gy) at the level of mouse survival, cellularity and progenitor ability. In support to our result, Mohrin et al. has found that both quiescent and proliferating adult mouse HSC were radioresistant being able to survive low doses of IR (2Gy). Mechanistically, quiescent HSC achieved their radioresistance by enhanced prosurvival gene expression (Mohrin et al., 2010). From another point of view, irradiation is one of the dangerous DNA damaging agents for HSC which are known to be one of the most radiosensitive tissues in the body. It is reported that total body irradiation (>4 Gy) leads to failure of HSCs while irradiation of HSCs at lower dose (2-3 Gy) differentially affected hematopoietic cells depending on their state of maturity and on their stage of ontogeny (Blanpain et al., 2011). Thus, it is quite possible that our stress was too strong on the HSC leading to its destruction and it is possible that if we use lower dose of irradiation (<2 Gy) we can see an altered stress-mediated response in *Xpc* KO cells. Furthermore, our data showed that *Xpc* KO did not affect the survival of mice treated with 5-FU. In consistence, Beerman et al. has showed that XPC expression decreases after induction of dormant HSC to enter into cycling state (Beerman et al., 2014). Finally, there might be other overlapping anti-apoptotic mechanisms in *Xpc* KO mice model that can compensate for loss of XPC.

Collectively, XPC did not show an effect on stem cell maintenance in murine hematopoiesis as we expected. However, we suggest that if we challenge the stem cell reconstitution capacity by performing a competitive transplantation assay, we will be able to determine an effect for XPC in HSC maintenance. For instance, Priestly et al. demonstrated that the effect of conditional excision of alpha4 integrin on the maintenance of HSC was very slight after primary transplantation, dominant after secondary transplantation and becomes lethal after tertiary transplantation (Priestley et al., 2006). Therefore, we assume that under regenerative stress, we can trace an effect for XPC on the maintenance of murine HSC.

III. General Conclusion

We have presented compelling evidence that XPC is modulated in hematopoietic cells according to the differentiation state. Our results on the functional study of XPC in human HSPC support the idea that XPC participates to the DNA survey in hematopoiesis, independently to the UV radiation, whereas this factor does not seem to be required for committed cell proliferation as initially suggested by the absent or low expression in CD34⁺ cells. Our data also show different XPC protein forms, due to unknown mechanism, certainly post-transcriptional modifications such as phosphorylation, which could be eventually linked to functional activity and resistance to stresses. Interestingly, it has been recently reported increased UV-sensitivity in leukemic cell lines after chemically forced differentiation with accumulation of DNA damages that are normally recognized by XPC. Therefore, our data might help to understand the potential implication of XPC in HSC and consequently leukemogenesis.

Nevertheless, our results on the functional study of XPC in mouse HSC have revealed that XPC did not exert effect on hematopoietic hemostasis in mouse BM. However *Xpc*-deficient cells have to be challenged by serial transplantations, which would better resemble to the human cell transplantation. One might also suggest that the variation in response of HSC to DNA damage depends on the stage of ontogeny. For instance, two recent studies by Milyavsky and Mohrin showed that the response to DNA damage differs between fetal HSC which die by apoptosis and adult HSC which survive by inducing DNA repair (Milyavsky et al., 2010; Mohrin et al., 2010). Another possibility could be that there exist inter-species specific differences in the DNA damage response in HSC that are collected from mouse or human.

The differences between the outcomes of the two studies (human and mouse) can be attributed to the knockout/knockdown strategies utilized since by the knockdown strategy the expression of the target gene is rapidly eliminated, while, by the knockout strategy, adaptation to the loss of the specific gene function might happen. In agreement with our speculation, Fong et al. used a knockdown strategy to show that XPC is fundamental for the maintenance of embryonic stem cells (Fong et al., 2011). However, Ito et al. used a knockout strategy of C-terminal region of *Xpc* in mouse ES cells to show that XPC is dispensable for maintenance of pluripotency and that XPC had no impact on self renewal of ES cells (Ito et al., 2014). Moreover, the difference between the outcomes of the human and mouse studies could be attributed to the distinctive role of XPC in the human and mouse HSC. It is apparent the XPC has an important function in the maintenance of the human HSC but, for the mouse HSC, it is quite possible that they may possess alternative pathways that compensate for XPC loss and preserve the maintenance of HSC.

Furthermore, studying the impact of aging on murine hematopoiesis, showed us that aging phenomenon lead to increase in stem cell amount (LSK, SLAM), decrease in progenitor cell amount (MPP) along with increase in progenitor ability of stem cells, increase in levels of monocytes, decrease in levels of B-lymphocytes in bone marrow, decrease in T-lymphocytes and increase in monocytes in peripheral blood. Moreover, oxidative stress increased in Lin⁻ cells with age. Conclusively, aging is associated with increase in stem cell pool, decrease lymphoid lineage and increase in myeloid lineage while XPC-defect is associated with increase in progenitor ability of stem cells but does not affect stem cell numbers or the hematopoietic composition.

IV. Perspectives

Overall, our *in vitro* studies support the high expression of XPC in CD34⁺ cells compared to the more committed CD34⁻ cells and that XPC deficiency has no effect on progenitors differentiation. Beside *in vivo* part, XPC expression in AML cell lines and its possible biochemical contribution in leukemia might be more clarified. Additionally, mass spectrometry analysis must be done in order to expose the origin of XPC three bands. Using NOD SCID mice engraftments approach; we showed the important role of XPC *in vivo* in maintenance of HSC. Consequently, this study has set the background for an extensive and generalized study of the various functional consequences and the underlying biochemical events that affect XPC-silenced HSC. Further molecular studies need to be done to investigate the pathway targeted during XPC deficiency that leads to diminution in stem cell levels.

XPC did not show an effect on stem cell maintenance in murine hematopoiesis as we expected. Although, we strongly postulate that if we challenge the stem cell activity to reconstitute the bone marrow in *Xpc* KO mice versus WT mice by doing serial transplantation assessing for the reconstitution potential (use CD45 marker for host-donor recognition-CD45.1 & CD45.2 alleles) we will see marked effect of XPC-deficiency on stem cell function. Thus, our aim will be to challenge the functional ability of stem cells to reconstitute the bone marrow by transplanting LSK cells from WT or *Xpc* KO mice and young or aged mice into irradiated Pepboy model. This will allow us to determine if XPC is important to function of stem cells in mice. We have done this experiment, unfortunately, our transplanted mice died because of gastrointestinal injuries due to extensive high irradiation of those mice. Only one mouse from each group survived so I have analyzed its peripheral blood to see whether the transplant worked well (**Figure 58**). Therefore, the surviving mice were well transplanted. Now we are re-doing the experiment in specialized laboratory in Paris. We have isolated Lin^- cells in our lab and we will send those cells for them to do the transplantation. Moreover, we can perform new experiments on *Xpc* KO mice subjecting them to lower doses of irradiation (<2Gy) where we will expect an altered response for XPC-defective cells to irradiation. Moreover, it is also possible to age more the *Xpc* KO mice in order to find an influence.

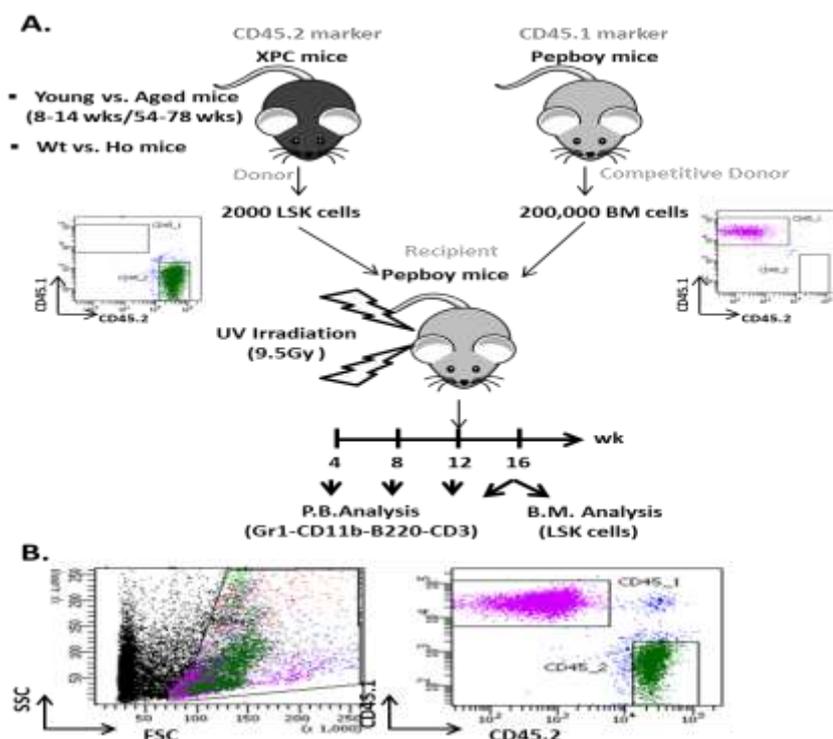


Figure 58. Long term repopulation ability of XPC-deficient stem cells. (A) Schematic representing procedure of competitive transplantation between *Xpc* KO mice (CD45.2 allelic marker) and Pepboy mice (CD45.1 allelic marker). Adjacent FACS graphs show allelic marker of each type of mice. PB was analyzed with time for identification of mature markers. (B) FACS image for LSK-transplanted Pepboy mice obtained from PB analysis after 3 weeks of transplant.

BIBLIOGRAPHY

- Abbotts, R., and Madhusudan, S. (2010). Human AP endonuclease 1 (APE1): From mechanistic insights to druggable target in cancer. *Cancer Treat. Rev.* *36*, 425–435.
- D'Adda di Fagagna, F. (2008). Living on a break: cellular senescence as a DNA-damage response. *Nat. Rev. Cancer* *8*, 512–522.
- Adimoolam, S., and Ford, J.M. (2002). p53 and DNA damage-inducible expression of the xeroderma pigmentosum group C gene. *Proc. Natl. Acad. Sci. U. S. A.* *99*, 12985–12990.
- Adolfsson, J., Månsson, R., Buza-Vidas, N., Hultquist, A., Liuba, K., Jensen, C.T., Bryder, D., Yang, L., Borge, O.-J., Thoren, L.A.M., et al. (2005). Identification of Flt3+ Lympho-Myeloid Stem Cells Lacking Erythro-Megakaryocytic Potential. *Cell* *121*, 295–306.
- Agarwal, S., Holton, K.L., and Lanza, R. (2008). Efficient differentiation of functional hepatocytes from human embryonic stem cells. *Stem Cells Dayt. Ohio* *26*, 1117–1127.
- Ahnesorg, P., Smith, P., and Jackson, S.P. (2006). XLF Interacts with the XRCC4-DNA Ligase IV Complex to Promote DNA Nonhomologous End-Joining. *Cell* *124*, 301–313.
- Akashi, K., Traver, D., Miyamoto, T., and Weissman, I.L. (2000). A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* *404*, 193–197.
- Allsopp, R.C., Cheshier, S., and Weissman, I.L. (2001). Telomere shortening accompanies increased cell cycle activity during serial transplantation of hematopoietic stem cells. *J. Exp. Med.* *193*, 917–924.
- Antonchuk, J., Sauvageau, G., and Humphries, R.K. (2002). HOXB4-induced expansion of adult hematopoietic stem cells ex vivo. *Cell* *109*, 39–45.
- Aoki, Y., Sato, A., Mizutani, S., and Takagi, M. (2014). Hematopoietic myeloid cell differentiation diminishes nucleotide excision repair. *Int. J. Hematol.*
- Arai, F., Hirao, A., Ohmura, M., Sato, H., Matsuoka, S., Takubo, K., Ito, K., Koh, G.Y., and Suda, T. (2004). Tie2/Angiopoietin-1 Signaling Regulates Hematopoietic Stem Cell Quiescence in the Bone Marrow Niche. *Cell* *118*, 149–161.
- Araki, M., Masutani, C., Takemura, M., Uchida, A., Sugasawa, K., Kondoh, J., Ohkuma, Y., and Hanaoka, F. (2001). Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair. *J. Biol. Chem.* *276*, 18665–18672.
- Araújo, S.J., Nigg, E.A., and Wood, R.D. (2001). Strong functional interactions of TFIIH with XPC and XPG in human DNA nucleotide excision repair, without a preassembled repairosome. *Mol. Cell. Biol.* *21*, 2281–2291.
- Arias, E.E., and Walter, J.C. (2007). Strength in numbers: preventing rereplication via multiple mechanisms in eukaryotic cells. *Genes Dev.* *21*, 497–518.
- Avasthi, S., Srivastava, R.N., and Singh, A. (2008). Stem Cell: Past, Present and Future- A Review Article. *Internet J. Med. Update - EJOURNAL* *3*.
- Babior, B.M. (1999). NADPH oxidase: an update. *Blood* *93*, 1464–1476.
- Babior, B.M. (2004). NADPH oxidase. *Curr. Opin. Immunol.* *16*, 42–47.

- Balaban, R.S., Nemoto, S., and Finkel, T. (2005). Mitochondria, Oxidants, and Aging. *Cell* 120, 483–495.
- Barker, J.N., and Wagner, J.E. (2003). Umbilical cord blood transplantation: current practice and future innovations. *Crit. Rev. Oncol. Hematol.* 48, 35–43.
- Barlow, C., Hirotsumi, S., Paylor, R., Liyanage, M., Eckhaus, M., Collins, F., Shiloh, Y., Crawley, J.N., Ried, T., Tagle, D., et al. (1996). Atm-deficient mice: a paradigm of ataxia telangiectasia. *Cell* 86, 159–171.
- Barnes, D.J., and Melo, J.V. (2006). Primitive, quiescent and difficult to kill: the role of non-proliferating stem cells in chronic myeloid leukemia. *Cell Cycle Georget. Tex* 5, 2862–2866.
- Barroca, V., Mouthon, M.A., Lewandowski, D., Brunet de la Grange, P., Gauthier, L.R., Pflumio, F., Boussin, F.D., Arwert, F., Riou, L., Allemand, I., et al. (2012). Impaired functionality and homing of Fancg-deficient hematopoietic stem cells. *Hum. Mol. Genet.* 21, 121–135.
- Bartek, J., and Lukas, J. (2007). DNA damage checkpoints: from initiation to recovery or adaptation. *Curr. Opin. Cell Biol.* 19, 238–245.
- Beerman, I., Seita, J., Inlay, M.A., Weissman, I.L., and Rossi, D.J. (2014). Quiescent Hematopoietic Stem Cells Accumulate DNA Damage during Aging that Is Repaired upon Entry into Cell Cycle. *Cell Stem Cell*.
- Behrens, A., van Deursen, J.M., Rudolph, K.L., and Schumacher, B. (2014). Impact of genomic damage and ageing on stem cell function. *Nat. Cell Biol.* 16, 201–207.
- Bender, C.F., Sikes, M.L., Sullivan, R., Huye, L.E., Le Beau, M.M., Roth, D.B., Mirzoeva, O.K., Oltz, E.M., and Petrini, J.H.J. (2002). Cancer predisposition and hematopoietic failure in Rad50(S/S) mice. *Genes Dev.* 16, 2237–2251.
- Bennett, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galton, D.A., Gralnick, H.R., and Sultan, C. (1976). Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. *Br. J. Haematol.* 33, 451–458.
- Bentley, D.J., Harrison, C., Ketchen, A.-M., Redhead, N.J., Samuel, K., Waterfall, M., Ansell, J.D., and Melton, D.W. (2002). DNA ligase I null mouse cells show normal DNA repair activity but altered DNA replication and reduced genome stability. *J. Cell Sci.* 115, 1551–1561.
- Benz, C., Copley, M.R., Kent, D.G., Wohrer, S., Cortes, A., Aghaeepour, N., Ma, E., Mader, H., Rowe, K., Day, C., et al. (2012). Hematopoietic Stem Cell Subtypes Expand Differentially during Development and Display Distinct Lymphopoietic Programs. *Cell Stem Cell* 10, 273–283.
- Berg, R.J., Ruven, H.J., Sands, A.T., de Gruijl, F.R., and Mullenders, L.H. (1998). Defective global genome repair in XPC mice is associated with skin cancer susceptibility but not with sensitivity to UVB induced erythema and edema. *J. Invest. Dermatol.* 110, 405–409.
- Bernardes de Jesus, B.M., Bjørås, M., Coin, F., and Egly, J.M. (2008). Dissection of the molecular defects caused by pathogenic mutations in the DNA repair factor XPC. *Mol. Cell. Biol.* 28, 7225–7235.

- Bertoncello, I., and Williams, B. (2004). Hematopoietic stem cell characterization by Hoechst 33342 and rhodamine 123 staining. *Methods Mol. Biol. Clifton NJ* 263, 181–200.
- Bhatia, M., Bonnet, D., Murdoch, B., Gan, O.I., and Dick, J.E. (1998). A newly discovered class of human hematopoietic cells with SCID-repopulating activity. *Nat. Med.* 4, 1038–1045.
- Blanpain, C., Mohrin, M., Sotiropoulou, P.A., and Passegué, E. (2011). DNA-damage response in tissue-specific and cancer stem cells. *Cell Stem Cell* 8, 16–29.
- Boer, J. de, and Hoeijmakers, J.H.J. (2000). Nucleotide excision repair and human syndromes. *Carcinogenesis* 21, 453–460.
- Boisset, J.-C., and Robin, C. (2012). On the origin of hematopoietic stem cells: progress and controversy. *Stem Cell Res.* 8, 1–13.
- Bokoch, G.M., and Knaus, U.G. (2003). NADPH oxidases: not just for leukocytes anymore! *Trends Biochem. Sci.* 28, 502–508.
- Bongso, A., and Tan, S. (2005). Human blastocyst culture and derivation of embryonic stem cell lines. *Stem Cell Rev.* 1, 87–98.
- Bonnet, D. (2002). Haematopoietic stem cells. *J. Pathol.* 197, 430–440.
- Boon, W.-M., Beissbarth, T., Hyde, L., Smyth, G., Gunnensen, J., Denton, D.A., Scott, H., and Tan, S.-S. (2004). A comparative analysis of transcribed genes in the mouse hypothalamus and neocortex reveals chromosomal clustering. *Proc. Natl. Acad. Sci. U. S. A.* 101, 14972–14977.
- Boonstra, J., and Post, J.A. (2004). Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells. *Gene* 337, 1–13.
- Bootsma, D., and Hoeijmakers, J.H. (1991). The genetic basis of xeroderma pigmentosum. *Ann. Génétique* 34, 143–150.
- Botta, E., Nardo, T., Orioli, D., Guglielmino, R., Ricotti, R., Bondanza, S., Benedicenti, F., Zambruno, G., and Stefanini, M. (2009). Genotype-phenotype relationships in trichothiodystrophy patients with novel splicing mutations in the XPD gene. *Hum. Mutat.* 30, 438–445.
- Bowie, M.B., McKnight, K.D., Kent, D.G., McCaffrey, L., Hoodless, P.A., and Eaves, C.J. (2006). Hematopoietic stem cells proliferate until after birth and show a reversible phase-specific engraftment defect. *J. Clin. Invest.* 116, 2808–2816.
- Bradford, G.B., Williams, B., Rossi, R., and Bertoncello, I. (1997). Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. *Exp. Hematol.* 25, 445–453.
- Bradley, T.R., and Metcalf, D. (1966). The growth of mouse bone marrow cells in vitro. *Aust. J. Exp. Biol. Med. Sci.* 44, 287–299.
- Brown, E.J., and Baltimore, D. (2000). ATR disruption leads to chromosomal fragmentation and early embryonic lethality. *Genes Dev.* 14, 397–402.
- Bryder, D., Rossi, D.J., and Weissman, I.L. (2006). Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *Am. J. Pathol.* 169, 338–346.

- Burke, B.A., and Carroll, M. (2010). BCR-ABL: a multi-faceted promoter of DNA mutation in chronic myelogenous leukemia. *Leukemia* 24, 1105–1112.
- Cadet, J., Douki, T., Gasparutto, D., and Ravanat, J.-L. (2003). Oxidative damage to DNA: formation, measurement and biochemical features. *Mutat. Res. Mol. Mech. Mutagen.* 531, 5–23.
- Calabretta, B., and Perrotti, D. (2004). The biology of CML blast crisis. *Blood* 103, 4010–4022.
- Caldecott, K.W. (2003). XRCC1 and DNA strand break repair. *DNA Repair* 2, 955–969.
- Calvi, L.M., Adams, G.B., Weibrecht, K.W., Weber, J.M., Olson, D.P., Knight, M.C., Martin, R.P., Schipani, E., Divieti, P., Bringhurst, F.R., et al. (2003). Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 425, 841–846.
- Canitrot, Y., Falinski, R., Louat, T., Laurent, G., Cazaux, C., Hoffmann, J.-S., Lautier, D., and Skorski, T. (2003). p210 BCR/ABL kinase regulates nucleotide excision repair (NER) and resistance to UV radiation. *Blood* 102, 2632–2637.
- Catlin, S.N., Busque, L., Gale, R.E., Gutter, P., and Abkowitz, J.L. (2011). The replication rate of human hematopoietic stem cells in vivo. *Blood* 117, 4460–4466.
- Lo Celso, C., Fleming, H.E., Wu, J.W., Zhao, C.X., Miake-Lye, S., Fujisaki, J., Côté, D., Rowe, D.W., Lin, C.P., and Scadden, D.T. (2009). Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. *Nature* 457, 92–96.
- Chambers, S.M., Shaw, C.A., Gatz, C., Fisk, C.J., Donehower, L.A., and Goodell, M.A. (2007). Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. *PLoS Biol.* 5, e201.
- Chandrasekhar, D., and Van Houten, B. (2000). In vivo formation and repair of cyclobutane pyrimidine dimers and 6-4 photoproducts measured at the gene and nucleotide level in *Escherichia coli*. *Mutat. Res.* 450, 19–40.
- Chang, D., Wang, F., Zhao, Y.-S., and Pan, H.-Z. (2008). Evaluation of oxidative stress in colorectal cancer patients. *Biomed. Environ. Sci.* BES 21, 286–289.
- Chen, Z., Yang, J., Wang, G., Song, B., Li, J., and Xu, Z. (2007). Attenuated expression of xeroderma pigmentosum group C is associated with critical events in human bladder cancer carcinogenesis and progression. *Cancer Res.* 67, 4578–4585.
- Cheo, D.L., Meira, L.B., Hammer, R.E., Burns, D.K., Doughty, A.T., and Friedberg, E.C. (1996). Synergistic interactions between XPC and p53 mutations in double-mutant mice: neural tube abnormalities and accelerated UV radiation-induced skin cancer. *Curr. Biol.* CB 6, 1691–1694.
- Cheo, D.L., Ruven, H.J., Meira, L.B., Hammer, R.E., Burns, D.K., Tappe, N.J., van Zeeland, A.A., Mullenders, L.H., and Friedberg, E.C. (1997). Characterization of defective nucleotide excision repair in XPC mutant mice. *Mutat. Res.* 374, 1–9.
- Cheo, D.L., Burns, D.K., Meira, L.B., Houle, J.F., and Friedberg, E.C. (1999). Mutational inactivation of the xeroderma pigmentosum group C gene confers predisposition to 2-acetylaminofluorene-induced liver and lung cancer and to spontaneous testicular cancer in Trp53^{-/-} mice. *Cancer Res.* 59, 771–775.

- Cheo, D.L., Meira, L.B., Burns, D.K., Reis, A.M., Issac, T., and Friedberg, E.C. (2000). Ultraviolet B radiation-induced skin cancer in mice defective in the Xpc, Trp53, and Apex (HAP1) genes: genotype-specific effects on cancer predisposition and pathology of tumors. *Cancer Res.* *60*, 1580–1584.
- Cheshier, S.H., Morrison, S.J., Liao, X., and Weissman, I.L. (1999). In vivo proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. *Proc. Natl. Acad. Sci. U. S. A.* *96*, 3120–3125.
- Cho, R.H., Sieburg, H.B., and Muller-Sieburg, C.E. (2008). A new mechanism for the aging of hematopoietic stem cells: aging changes the clonal composition of the stem cell compartment but not individual stem cells. *Blood* *111*, 5553–5561.
- Chow, A., Lucas, D., Hidalgo, A., Méndez-Ferrer, S., Hashimoto, D., Scheiermann, C., Battista, M., Leboeuf, M., Prophete, C., van Rooijen, N., et al. (2011). Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. *J. Exp. Med.* *208*, 261–271.
- Chow, D.C., Wenning, L.A., Miller, W.M., and Papoutsakis, E.T. (2001). Modeling pO₂ Distributions in the Bone Marrow Hematopoietic Compartment. II. Modified Kroghian Models. *Biophys. J.* *81*, 685–696.
- Cipolleschi, M.G., Dello Sbarba, P., and Olivotto, M. (1993). The role of hypoxia in the maintenance of hematopoietic stem cells. *Blood* *82*, 2031–2037.
- Clarke, M.F., and Fuller, M. (2006). Stem cells and cancer: two faces of eve. *Cell* *124*, 1111–1115.
- Cleaver, J.E. (2005a). Cancer in xeroderma pigmentosum and related disorders of DNA repair. *Nat. Rev. Cancer* *5*, 564–573.
- Cleaver, J.E. (2005b). Cancer in xeroderma pigmentosum and related disorders of DNA repair. *Nat. Rev. Cancer* *5*, 564–573.
- Cleaver, J.E., Lam, E.T., and Revet, I. (2009). Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. *Nat. Rev. Genet.* *10*, 756–768.
- Clement, F.C., Camenisch, U., Fei, J., Kaczmarek, N., Mathieu, N., and Naegeli, H. (2010). Dynamic two-stage mechanism of versatile DNA damage recognition by xeroderma pigmentosum group C protein. *Mutat. Res. Mol. Mech. Mutagen.* *685*, 21–28.
- Colella, S., Nardo, T., Botta, E., Lehmann, A.R., and Stefanini, M. (2000). Identical mutations in the CSB gene associated with either Cockayne syndrome or the DeSanctis-cacchione variant of xeroderma pigmentosum. *Hum. Mol. Genet.* *9*, 1171–1175.
- Consortium, I.H.G.S. (2004). Finishing the euchromatic sequence of the human genome. *Nature* *431*, 931–945.
- Cox, J.A., Jeng, A.Y., Blumberg, P.M., and Tauber, A.I. (1987). Comparison of subcellular activation of the human neutrophil NADPH-oxidase by arachidonic acid, sodium dodecyl sulfate (SDS), and phorbol myristate acetate (PMA). *J. Immunol.* *138*, 1884–1888.
- Cramer, K., Nieborowska-Skorska, M., Koptyra, M., Slupianek, A., Penserga, E.T.P., Eaves, C.J., Aulitzky, W., and Skorski, T. (2008). BCR/ABL and other kinases from chronic

- myeloproliferative disorders stimulate single-strand annealing, an unfaithful DNA double-strand break repair. *Cancer Res.* *68*, 6884–6888.
- Cross, A.R., and Segal, A.W. (2004). The NADPH oxidase of professional phagocytes—prototype of the NOX electron transport chain systems. *Biochim. Biophys. Acta BBA - Bioenerg.* *1657*, 1–22.
- Daboussi, F., Zaslavskiy, M., Poirot, L., Loperfido, M., Gouble, A., Guyot, V., Leduc, S., Galetto, R., Grizot, S., Oficjalska, D., et al. (2012). Chromosomal context and epigenetic mechanisms control the efficacy of genome editing by rare-cutting designer endonucleases. *Nucleic Acids Res.* *40*, 6367–6379.
- Dai, Q.-S., Hua, R.-X., Zeng, R.-F., Long, J.-T., and Peng, Z.-W. (2014). XPC gene polymorphisms contribute to bladder cancer susceptibility: a meta-analysis. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* *35*, 447–453.
- Danet, G.H., Pan, Y., Luongo, J.L., Bonnet, D.A., and Simon, M.C. (2003). Expansion of human SCID-repopulating cells under hypoxic conditions. *J. Clin. Invest.* *112*, 126–135.
- DeFazio, L.G., Stansel, R.M., Griffith, J.D., and Chu, G. (2002). Synapsis of DNA ends by DNA-dependent protein kinase. *EMBO J.* *21*, 3192–3200.
- D’Errico, M., Teson, M., Calcagnile, A., Nardo, T., De Luca, N., Lazzari, C., Soddu, S., Zambruno, G., Stefanini, M., and Dogliotti, E. (2005). Differential role of transcription-coupled repair in UVB-induced response of human fibroblasts and keratinocytes. *Cancer Res.* *65*, 432–438.
- D’Errico, M., Parlanti, E., Teson, M., de Jesus, B.M.B., Degan, P., Calcagnile, A., Jaruga, P., Bjørås, M., Crescenzi, M., Pedrini, A.M., et al. (2006). New functions of XPC in the protection of human skin cells from oxidative damage. *EMBO J.* *25*, 4305–4315.
- Deutsch, E., Dugray, A., AbdulKarim, B., Marangoni, E., Maggiorella, L., Vaganay, S., M’Kacher, R., Rasy, S.D., Eschwege, F., Vainchenker, W., et al. (2001). BCR-ABL down-regulates the DNA repair protein DNA-PKcs. *Blood* *97*, 2084–2090.
- Dierov, J., Sanchez, P.V., Burke, B.A., Padilla-Nash, H., Putt, M.E., Ried, T., and Carroll, M. (2009). BCR/ABL induces chromosomal instability after genotoxic stress and alters the cell death threshold. *Leukemia* *23*, 279–286.
- Dip, R., Camenisch, U., and Naegeli, H. (2004). Mechanisms of DNA damage recognition and strand discrimination in human nucleotide excision repair. *DNA Repair* *3*, 1409–1423.
- Doulatov, S., Notta, F., Laurenti, E., and Dick, J.E. (2012). Hematopoiesis: a human perspective. *Cell Stem Cell* *10*, 120–136.
- Draenert, K., and Draenert, Y. (1980). The vascular system of bone marrow. *Scan. Electron Microsc.* 113–122.
- Druker, B.J., Guilhot, F., O’Brien, S.G., Gathmann, I., Kantarjian, H., Gattermann, N., Deininger, M.W.N., Silver, R.T., Goldman, J.M., Stone, R.M., et al. (2006). Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N. Engl. J. Med.* *355*, 2408–2417.

- Dumble, M., Moore, L., Chambers, S.M., Geiger, H., Van Zant, G., Goodell, M.A., and Donehower, L.A. (2007). The impact of altered p53 dosage on hematopoietic stem cell dynamics during aging. *Blood* *109*, 1736–1742.
- Dupuy, A., Valton, J., Leduc, S., Armier, J., Galetto, R., Gouble, A., Lebuhotel, C., Sary, A., Pâques, F., Duchateau, P., et al. (2013). Targeted Gene Therapy of Xeroderma Pigmentosum Cells Using Meganuclease and TALENTM. *PLoS ONE* *8*, e78678.
- Dykstra, B., Kent, D., Bowie, M., McCaffrey, L., Hamilton, M., Lyons, K., Lee, S.-J., Brinkman, R., and Eaves, C. (2007). Long-Term Propagation of Distinct Hematopoietic Differentiation Programs In Vivo. *Cell Stem Cell* *1*, 218–229.
- Elrick, L.J., Jorgensen, H.G., Mountford, J.C., and Holyoake, T.L. (2005). Punish the parent not the progeny. *Blood* *105*, 1862–1866.
- Ema, H., Takano, H., Sudo, K., and Nakauchi, H. (2000). In Vitro Self-Renewal Division of Hematopoietic Stem Cells. *J. Exp. Med.* *192*, 1281–1288.
- Ema, H., Morita, Y., and Suda, T. (2014). Heterogeneity and hierarchy of hematopoietic stem cells. *Exp. Hematol.* *42*, 74–82.e2.
- Engelhardt, M., Kumar, R., Albanell, J., Pettengell, R., Han, W., and Moore, M.A. (1997). Telomerase regulation, cell cycle, and telomere stability in primitive hematopoietic cells. *Blood* *90*, 182–193.
- English, J.S., and Swerdlow, A.J. (1987). The risk of malignant melanoma, internal malignancy and mortality in xeroderma pigmentosum patients. *Br. J. Dermatol.* *117*, 457–461.
- Epstein, A.C.R., Gleadle, J.M., McNeill, L.A., Hewitson, K.S., O'Rourke, J., Mole, D.R., Mukherji, M., Metzen, E., Wilson, M.I., Dhanda, A., et al. (2001). *C. elegans* EGL-9 and Mammalian Homologs Define a Family of Dioxygenases that Regulate HIF by Prolyl Hydroxylation. *Cell* *107*, 43–54.
- Ergün, S.S., Cek, D.I., and Demirkesen, C. (2002). Is facial resurfacing with monobloc full-thickness skin graft a remedy in xeroderma pigmentosum? *Plast. Reconstr. Surg.* *110*, 1290–1293.
- Evans, M.J., and Kaufman, M.H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* *292*, 154–156.
- Faghri, S., Tamura, D., Kraemer, K.H., and Digiovanna, J.J. (2008). Trichothiodystrophy: a systematic review of 112 published cases characterises a wide spectrum of clinical manifestations. *J. Med. Genet.* *45*, 609–621.
- Faiola, B., Fuller, E.S., Wong, V.A., Pluta, L., Abernethy, D.J., Rose, J., and Recio, L. (2004). Exposure of hematopoietic stem cells to benzene or 1,4-benzoquinone induces gender-specific gene expression. *Stem Cells Dayt. Ohio* *22*, 750–758.
- Fan, J., Li, L., Small, D., and Rassool, F. (2010). Cells expressing FLT3/ITD mutations exhibit elevated repair errors generated through alternative NHEJ pathways: implications for genomic instability and therapy. *Blood* *116*, 5298–5305.
- Fedoročko, P., Macková, N.O., Šándorčínová, Z., Sedláková-Hoferová, Z., Solár, P., and Chlebovský, O. (2000). Influence of age and K, Mg aspartate (Cardilan) on murine haemopoiesis. *Mech. Ageing Dev.* *119*, 159–170.

- Fernandes, M.S., Reddy, M.M., Gonneville, J.R., DeRoo, S.C., Podar, K., Griffin, J.D., Weinstock, D.M., and Sattler, M. (2009). BCR-ABL promotes the frequency of mutagenic single-strand annealing DNA repair. *Blood* 114, 1813–1819.
- Finkel, T. (2003). Oxidant signals and oxidative stress. *Curr. Opin. Cell Biol.* 15, 247–254.
- Fischer, A., and Cavazzana-Calvo, M. (2005). Integration of retroviruses: a fine balance between efficiency and danger. *PLoS Med.* 2, e10.
- Fischer, J.L., Kumar, M.A.S., Day, T.W., Hardy, T.M., Hamilton, S., Besch-Williford, C., Safa, A.R., Pollok, K.E., and Smith, M.L. (2009). The Xpc gene markedly affects cell survival in mouse bone marrow. *Mutagenesis* 24, 309–316.
- Fisher, A.M., Danenberg, K., Banerjee, D., Bertino, J.R., Danenberg, P., and Gomer, C.J. (1997). Increased photosensitivity in HL60 cells expressing wild-type p53. *Photochem. Photobiol.* 66, 265–270.
- Folz, R.J., and Crapo, J.D. (1994). Extracellular Superoxide Dismutase (SOD3): Tissue-Specific Expression, Genomic Characterization, and Computer-Assisted Sequence Analysis of the Human EC SOD Gene. *Genomics* 22, 162–171.
- Fong, Y.W., Inouye, C., Yamaguchi, T., Cattoglio, C., Grubisic, I., and Tjian, R. (2011). A DNA repair complex functions as an Oct4/Sox2 coactivator in embryonic stem cells. *Cell* 147, 120–131.
- Ford, J.M., and Hanawalt, P.C. (1995). Li-Fraumeni syndrome fibroblasts homozygous for p53 mutations are deficient in global DNA repair but exhibit normal transcription-coupled repair and enhanced UV resistance. *Proc. Natl. Acad. Sci. U. S. A.* 92, 8876–8880.
- Ford, J.M., and Hanawalt, P.C. (1997). Expression of wild-type p53 is required for efficient global genomic nucleotide excision repair in UV-irradiated human fibroblasts. *J. Biol. Chem.* 272, 28073–28080.
- Forget, A.L., and Kowalczykowski, S.C. (2010). Single-molecule imaging brings Rad51 nucleoprotein filaments into focus. *Trends Cell Biol.* 20, 269–276.
- Fortini, P., and Dogliotti, E. (2007). Base damage and single-strand break repair: Mechanisms and functional significance of short- and long-patch repair subpathways. *DNA Repair* 6, 398–409.
- Foudi, A., Hochedlinger, K., Van Buren, D., Schindler, J.W., Jaenisch, R., Carey, V., and Hock, H. (2009). Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. *Nat. Biotechnol.* 27, 84–90.
- Fréchet, M., Warrick, E., Vioux, C., Chevallier, O., Spatz, A., Benhamou, S., Sarasin, A., Bernerd, F., and Magnaldo, T. (2008a). Overexpression of matrix metalloproteinase 1 in dermal fibroblasts from DNA repair-deficient/cancer-prone xeroderma pigmentosum group C patients. *Oncogene* 27, 5223–5232.
- Fréchet, M., Warrick, E., Vioux, C., Chevallier, O., Spatz, A., Benhamou, S., Sarasin, A., Bernerd, F., and Magnaldo, T. (2008b). Overexpression of matrix metalloproteinase 1 in dermal fibroblasts from DNA repair-deficient/cancer-prone xeroderma pigmentosum group C patients. *Oncogene* 27, 5223–5232.

- Friedberg, E.C. (1995). Out of the shadows and into the light: the emergence of DNA repair. *Trends Biochem. Sci.* 20, 381.
- Friedberg, E.C. (2001). How nucleotide excision repair protects against cancer. *Nat. Rev. Cancer* 1, 22–33.
- Friedberg, E.C., Walker, G.C., and Siede, W. (1995). *DNA Repair and Mutagenesis* (ASM Press).
- Friedberg, E.C., Cheo, D.L., Meira, L.B., and Reis, A.M. (1999). Cancer predisposition in mutant mice defective in the XPC DNA repair gene. *Prog. Exp. Tumor Res.* 35, 37–52.
- Friedberg, E.C., Bond, J.P., Burns, D.K., Cheo, D.L., Greenblatt, M.S., Meira, L.B., Nahari, D., and Reis, A.M. (2000). Defective nucleotide excision repair in xpc mutant mice and its association with cancer predisposition. *Mutat. Res.* 459, 99–108.
- Friedberg, E.C., Aguilera, A., Gellert, M., Hanawalt, P.C., Hays, J.B., Lehmann, A.R., Lindahl, T., Lowndes, N., Sarasin, A., and Wood, R.D. (2006). DNA repair: from molecular mechanism to human disease. *DNA Repair* 5, 986–996.
- Fuchs, E., and Horsley, V. (2011). Ferreting out stem cells from their niches. *Nat. Cell Biol.* 13, 513–518.
- Galio, L., Bouquet, C., and Brooks, P. (1999). ATP hydrolysis-dependent formation of a dynamic ternary nucleoprotein complex with MutS and MutL. *Nucleic Acids Res.* 27, 2325–2331.
- Geiszt, M., and Leto, T.L. (2004). The Nox Family of NAD(P)H Oxidases: Host Defense and Beyond. *J. Biol. Chem.* 279, 51715–51718.
- Giglia, G., Dumaz, N., Drougard, C., Avril, M.F., Daya-Grosjean, L., and Sarasin, A. (1998). p53 mutations in skin and internal tumors of xeroderma pigmentosum patients belonging to the complementation group C. *Cancer Res.* 58, 4402–4409.
- Giglia-Mari, G., Coin, F., Ranish, J.A., Hoogstraten, D., Theil, A., Wijgers, N., Jaspers, N.G.J., Raams, A., Argentini, M., van der Spek, P.J., et al. (2004). A new, tenth subunit of TFIIH is responsible for the DNA repair syndrome trichothiodystrophy group A. *Nat. Genet.* 36, 714–719.
- Goessler, U.R., Riedel, K., Hormann, K., and Riedel, F. (2006). Perspectives of gene therapy in stem cell tissue engineering. *Cells Tissues Organs* 183, 169–179.
- Gonzalez-Huici, V., Szakal, B., Urulangodi, M., Psakhye, I., Castellucci, F., Menolfi, D., Rajakumara, E., Fumasoni, M., Bermejo, R., Jentsch, S., et al. (2014). DNA bending facilitates the error-free DNA damage tolerance pathway and upholds genome integrity. *EMBO J.* 33, 327–340.
- Goodell, M.A., Brose, K., Paradis, G., Conner, A.S., and Mulligan, R.C. (1996). Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J. Exp. Med.* 183, 1797–1806.
- Goosen, N. (2010). Scanning the DNA for damage by the nucleotide excision repair machinery. *DNA Repair* 9, 593–596.

- Gram, G.J., Nielsen, S.D., and Hansen, J.E. (1998). Spontaneous silencing of humanized green fluorescent protein (hGFP) gene expression from a retroviral vector by DNA methylation. *J. Hematother.* 7, 333–341.
- Greer, E.L., and Brunet, A. (2005). FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene* 24, 7410–7425.
- Grewal, S.S., Kahn, J.P., MacMillan, M.L., Ramsay, N.K.C., and Wagner, J.E. (2004). Successful hematopoietic stem cell transplantation for Fanconi anemia from an unaffected HLA-genotype-identical sibling selected using preimplantation genetic diagnosis. *Blood* 103, 1147–1151.
- Gu, C., Zhang, Q., Yang, Z., Wang, Y., Zou, Y., and Wang, Y. (2006). Recognition and incision of oxidative intrastrand cross-link lesions by UvrABC nuclease. *Biochemistry (Mosc.)* 45, 10739–10746.
- Guan, Y., Gerhard, B., and Hogge, D.E. (2003). Detection, isolation, and stimulation of quiescent primitive leukemic progenitor cells from patients with acute myeloid leukemia (AML). *Blood* 101, 3142–3149.
- Guilak, F., Estes, B.T., Diekman, B.O., Moutos, F.T., and Gimble, J.M. (2010). 2010 Nicolas Andry Award: Multipotent adult stem cells from adipose tissue for musculoskeletal tissue engineering. *Clin. Orthop.* 468, 2530–2540.
- Guillem, V.M., Cervantes, F., Martínez, J., Alvarez-Larrán, A., Collado, M., Camós, M., Sureda, A., Maffioli, M., Marugán, I., and Hernández-Boluda, J.-C. (2010a). XPC genetic polymorphisms correlate with the response to imatinib treatment in patients with chronic phase chronic myeloid leukemia. *Am. J. Hematol.* 85, 482–486.
- Guillem, V.M., Cervantes, F., Martínez, J., Alvarez-Larrán, A., Collado, M., Camós, M., Sureda, A., Maffioli, M., Marugán, I., and Hernández-Boluda, J.-C. (2010b). XPC genetic polymorphisms correlate with the response to imatinib treatment in patients with chronic phase chronic myeloid leukemia. *Am. J. Hematol.* 85, 482–486.
- Guillotin, D., and Martin, S.A. (2014). Exploiting DNA mismatch repair deficiency as a therapeutic strategy. *Exp. Cell Res.* 329, 110–115.
- Guo, Y., Lübbert, M., and Engelhardt, M. (2003). CD34- hematopoietic stem cells: current concepts and controversies. *Stem Cells Dayt. Ohio* 21, 15–20.
- Guo, Z., Kozlov, S., Lavin, M.F., Person, M.D., and Paull, T.T. (2010). ATM Activation by Oxidative Stress. *Science* 330, 517–521.
- Guzman, M.L., and Jordan, C.T. (2009). Lessons learned from the study of JunB: new insights for normal and leukemia stem cell biology. *Cancer Cell* 15, 252–254.
- De Haan, G., Nijhof, W., and Van Zant, G. (1997). Mouse strain-dependent changes in frequency and proliferation of hematopoietic stem cells during aging: correlation between lifespan and cycling activity. *Blood* 89, 1543–1550.
- Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M.P., Wulffraat, N., Leboulch, P., Lim, A., Osborne, C.S., Pawliuk, R., Morillon, E., et al. (2003). LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 302, 415–419.

- Hakem, R. (2008). DNA-damage repair; the good, the bad, and the ugly. *EMBO J.* 27, 589–605.
- Hananian, J., and Cleaver, J.E. (1980). Xeroderma pigmentosum exhibiting neurological disorders and systemic lupus erythematosus. *Clin. Genet.* 17, 39–45.
- Hanawalt, P.C. (2002). Subpathways of nucleotide excision repair and their regulation. *Oncogene* 21, 8949–8956.
- Haneline, L.S., Gobbett, T.A., Ramani, R., Carreau, M., Buchwald, M., Yoder, M.C., and Clapp, D.W. (1999). Loss of FancC function results in decreased hematopoietic stem cell repopulating ability. *Blood* 94, 1–8.
- Harman, D. (1956). Aging: A Theory Based on Free Radical and Radiation Chemistry. *J. Gerontol.* 11, 298–300.
- Hasty, P., Campisi, J., Hoeijmakers, J., van Steeg, H., and Vijg, J. (2003). Aging and genome maintenance: lessons from the mouse? *Science* 299, 1355–1359.
- Hayflick, L. (1976). The cell biology of human aging. *N. Engl. J. Med.* 295, 1302–1308.
- He, J., Shi, T.-Y., Zhu, M.-L., Wang, M.-Y., Li, Q.-X., and Wei, Q.-Y. (2013). Associations of Lys939Gln and Ala499Val polymorphisms of the XPC gene with cancer susceptibility: a meta-analysis. *Int. J. Cancer J. Int. Cancer* 133, 1765–1775.
- Henning, K.A., Li, L., Iyer, N., McDaniel, L.D., Reagan, M.S., Legerski, R., Schultz, R.A., Stefanini, M., Lehmann, A.R., Mayne, L.V., et al. (1995). The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIF. *Cell* 82, 555–564.
- Herbig, U., Jobling, W.A., Chen, B.P.C., Chen, D.J., and Sedivy, J.M. (2004). Telomere Shortening Triggers Senescence of Human Cells through a Pathway Involving ATM, p53, and p21CIP1, but Not p16INK4a. *Mol. Cell* 14, 501–513.
- Hermanson-Miller, I.L., and Turchi, J.J. (2002). Strand-Specific Binding of RPA and XPA to Damaged Duplex DNA†. *Biochemistry (Mosc.)* 41, 2402–2408.
- Hess, M.T., Schwitter, U., Petretta, M., Giese, B., and Naegeli, H. (1997). Bipartite substrate discrimination by human nucleotide excision repair. *Proc. Natl. Acad. Sci.* 94, 6664–6669.
- Hitomi, K., Iwai, S., and Tainer, J.A. (2007). The intricate structural chemistry of base excision repair machinery: Implications for DNA damage recognition, removal, and repair. *DNA Repair* 6, 410–428.
- Hoeijmakers, J.H.J. (2009). DNA damage, aging, and cancer. *N. Engl. J. Med.* 361, 1475–1485.
- Hollander, M.C., Philburn, R.T., Patterson, A.D., Velasco-Miguel, S., Friedberg, E.C., Linnoila, R.I., and Fornace, A.J., Jr (2005a). Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 102, 13200–13205.
- Hollander, M.C., Philburn, R.T., Patterson, A.D., Velasco-Miguel, S., Friedberg, E.C., Linnoila, R.I., and Fornace, A.J., Jr (2005b). Deletion of XPC leads to lung tumors in mice

- and is associated with early events in human lung carcinogenesis. *Proc. Natl. Acad. Sci. U. S. A.* *102*, 13200–13205.
- Hoogstraten, D., Bergink, S., Ng, J.M.Y., Verbiest, V.H.M., Luijsterburg, M.S., Geverts, B., Raams, A., Dinant, C., Hoeijmakers, J.H.J., Vermeulen, W., et al. (2008). Versatile DNA damage detection by the global genome nucleotide excision repair protein XPC. *J. Cell Sci.* *121*, 2850–2859.
- Horibata, K., Iwamoto, Y., Kuraoka, I., Jaspers, N.G.J., Kurimasa, A., Oshimura, M., Ichihashi, M., and Tanaka, K. (2004). Complete absence of Cockayne syndrome group B gene product gives rise to UV-sensitive syndrome but not Cockayne syndrome. *Proc. Natl. Acad. Sci. U. S. A.* *101*, 15410–15415.
- Hsu, P., Hanawalt, P.C., and Nospikel, T. (2007). Nucleotide excision repair phenotype of human acute myeloid leukemia cell lines at various stages of differentiation. *Mutat. Res.* *614*, 3–15.
- Hu, J., McCall, C.M., Ohta, T., and Xiong, Y. (2004). Targeted ubiquitination of CDT1 by the DDB1-CUL4A-ROC1 ligase in response to DNA damage. *Nat. Cell Biol.* *6*, 1003–1009.
- Hu, Z., Wang, Y., Wang, X., Liang, G., Miao, X., Xu, Y., Tan, W., Wei, Q., Lin, D., and Shen, H. (2005). DNA repair gene XPC genotypes/haplotypes and risk of lung cancer in a Chinese population. *Int. J. Cancer J. Int. Cancer* *115*, 478–483.
- Hui, H., Tang, Y., Hu, M., and Zhao, X. (2011). Stem Cells: General Features and Characteristics. In *Stem Cells in Clinic and Research*, A. Gholamrezanezhad, ed. (InTech),.
- Hwang, B.J., Ford, J.M., Hanawalt, P.C., and Chu, G. (1999). Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. *Proc. Natl. Acad. Sci. U. S. A.* *96*, 424–428.
- Hyka-Nospikel, N., Lemonidis, K., Lu, W.-T., and Nospikel, T. (2011). Circulating human B lymphocytes are deficient in nucleotide excision repair and accumulate mutations upon proliferation. *Blood* *117*, 6277–6286.
- Ikuta, K., and Weissman, I.L. (1992). Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. *Proc. Natl. Acad. Sci. U. S. A.* *89*, 1502–1506.
- Inaba, M., and Yamashita, Y.M. (2012). Asymmetric stem cell division: precision for robustness. *Cell Stem Cell* *11*, 461–469.
- Inlay, M.A., Bhattacharya, D., Sahoo, D., Serwold, T., Seita, J., Karsunky, H., Plevritis, S.K., Dill, D.L., and Weissman, I.L. (2009). Ly6d marks the earliest stage of B-cell specification and identifies the branchpoint between B-cell and T-cell development. *Genes Dev.* *23*, 2376–2381.
- Inomata, K., Aoto, T., Binh, N.T., Okamoto, N., Tanimura, S., Wakayama, T., Iseki, S., Hara, E., Masunaga, T., Shimizu, H., et al. (2009). Genotoxic stress abrogates renewal of melanocyte stem cells by triggering their differentiation. *Cell* *137*, 1088–1099.
- Ip, S.C.Y., Rass, U., Blanco, M.G., Flynn, H.R., Skehel, J.M., and West, S.C. (2008). Identification of Holliday junction resolvases from humans and yeast. *Nature* *456*, 357–361.

- Ishikawa, F., Yoshida, S., Saito, Y., Hijikata, A., Kitamura, H., Tanaka, S., Nakamura, R., Tanaka, T., Tomiyama, H., Saito, N., et al. (2007). Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nat. Biotechnol.* *25*, 1315–1321.
- Itin, P.H., Sarasin, A., and Pittelkow, M.R. (2001). Trichothiodystrophy: update on the sulfur-deficient brittle hair syndromes. *J. Am. Acad. Dermatol.* *44*, 891–920; quiz 921–924.
- Ito, K., Hirao, A., Arai, F., Matsuoka, S., Takubo, K., Hamaguchi, I., Nomiyama, K., Hosokawa, K., Sakurada, K., Nakagata, N., et al. (2004a). Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature* *431*, 997–1002.
- Ito, K., Hirao, A., Arai, F., Matsuoka, S., Takubo, K., Hamaguchi, I., Nomiyama, K., Hosokawa, K., Sakurada, K., Nakagata, N., et al. (2004b). Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature* *431*, 997–1002.
- Ito, K., Hirao, A., Arai, F., Takubo, K., Matsuoka, S., Miyamoto, K., Ohmura, M., Naka, K., Hosokawa, K., Ikeda, Y., et al. (2006). Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat. Med.* *12*, 446–451.
- Ito, S., Yamane, M., Ohtsuka, S., and Niwa, H. (2014). The C-terminal region of Xpc is dispensable for the transcriptional activity of Oct3/4 in mouse embryonic stem cells. *FEBS Lett.* *588*, 1128–1135.
- Ivanovic, Z., Hermitte, F., de la Grange, P.B., Dazey, B., Belloc, F., Lacombe, F., Vezon, G., and Praloran, V. (2004). Simultaneous Maintenance of Human Cord Blood SCID-Repopulating Cells and Expansion of Committed Progenitors at Low O₂ Concentration (3%). *STEM CELLS* *22*, 716–724.
- Ivanovic, Z., Duchez, P., Morgan, D.A., Hermitte, F., Lafarge, X., Chevaleyre, J., Praloran, V., Dazey, B., Vezon, G., and Boiron, J.-M. (2006). Whole-blood leuko-depletion filters as a source of CD 34+ progenitors potentially usable in cell therapy. *Transfusion (Paris)* *46*, 118–125.
- Iyer, N.V., Kotch, L.E., Agani, F., Leung, S.W., Laughner, E., Wenger, R.H., Gassmann, M., Gearhart, J.D., Lawler, A.M., Yu, A.Y., et al. (1998). Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 α . *Genes Dev.* *12*, 149–162.
- Jeggo, P., and O'Neill, P. (2002). The Greek Goddess, Artemis, reveals the secrets of her cleavage. *DNA Repair* *1*, 771–777.
- Jenkinson, E.J., Anderson, G., and Owen, J.J. (1992). Studies on T cell maturation on defined thymic stromal cell populations in vitro. *J. Exp. Med.* *176*, 845–853.
- Jiang, X., Zhou, L.-T., Zhang, S.-C., and Chen, K. (2012). XPC Polymorphism Increases Risk of Digestive System Cancers: Current Evidence from A Meta-Analysis. *Chin. J. Cancer Res. Chung-Kuo Yen Cheng Yen Chiu* *24*, 181–189.
- Jin, B., Dong, Y., Zhang, X., Wang, H., and Han, B. (2014). Association of XPC Polymorphisms and Lung Cancer Risk: A Meta-Analysis. *PloS One* *9*, e93937.
- Jing, D., Fonseca, A.-V., Alakel, N., Fierro, F.A., Muller, K., Bornhauser, M., Ehninger, G., Corbeil, D., and Ordemann, R. (2010). Hematopoietic stem cells in co-culture with

- mesenchymal stromal cells--modeling the niche compartments in vitro. *Haematologica* 95, 542–550.
- Jones, R.J., Collector, M.I., Barber, J.P., Vala, M.S., Fackler, M.J., May, W.S., Griffin, C.A., Hawkins, A.L., Zehnbauer, B.A., Hilton, J., et al. (1996). Characterization of mouse lymphohematopoietic stem cells lacking spleen colony-forming activity. *Blood* 88, 487–491.
- Jordan, C.T., and Lemischka, I.R. (1990). Clonal and systemic analysis of long-term hematopoiesis in the mouse. *Genes Dev.* 4, 220–232.
- Jordan, C.T., Guzman, M.L., and Noble, M. (2006). Cancer stem cells. *N. Engl. J. Med.* 355, 1253–1261.
- Kadyrov, F.A., Holmes, S.F., Arana, M.E., Lukianova, O.A., O'Donnell, M., Kunkel, T.A., and Modrich, P. (2007). *Saccharomyces cerevisiae* MutL α Is a Mismatch Repair Endonuclease. *J. Biol. Chem.* 282, 37181–37190.
- Kaelin Jr., W.G., and Ratcliffe, P.J. (2008). Oxygen Sensing by Metazoans: The Central Role of the HIF Hydroxylase Pathway. *Mol. Cell* 30, 393–402.
- Kamata, H., and Hirata, H. (1999). Redox Regulation of Cellular Signalling. *Cell. Signal.* 11, 1–14.
- Kassam, S.N., and Rainbow, A.J. (2007). Deficient base excision repair of oxidative DNA damage induced by methylene blue plus visible light in xeroderma pigmentosum group C fibroblasts. *Biochem. Biophys. Res. Commun.* 359, 1004–1009.
- Katsura, Y., and Kawamoto, H. (2001). Stepwise Lineage Restriction of Progenitors in Lympho-Myelopoiesis. *Int. Rev. Immunol.* 20, 1–20.
- Kawamoto, H., Ohmura, K., and Katsura, Y. (1997). Direct evidence for the commitment of hematopoietic stem cells to T, B and myeloid lineages in murine fetal liver. *Int. Immunol.* 9, 1011–1019.
- Kawamoto, H., Ohmura, K., Fujimoto, S., and Katsura, Y. (1999). Emergence of T cell progenitors without B cell or myeloid differentiation potential at the earliest stage of hematopoiesis in the murine fetal liver. *J. Immunol. Baltim. Md 1950* 162, 2725–2731.
- Kenyon, J., and Gerson, S.L. (2007). The role of DNA damage repair in aging of adult stem cells. *Nucleic Acids Res.* 35, 7557–7565.
- Khan, S.G., Levy, H.L., Legerski, R., Quackenbush, E., Reardon, J.T., Emmert, S., Sancar, A., Li, L., Schneider, T.D., Cleaver, J.E., et al. (1998). Xeroderma pigmentosum group C splice mutation associated with autism and hypoglycinemia. *J. Invest. Dermatol.* 111, 791–796.
- Khan, S.G., Metin, A., Gozukara, E., Inui, H., Shahlavi, T., Muniz-Medina, V., Baker, C.C., Ueda, T., Aiken, J.R., Schneider, T.D., et al. (2004). Two essential splice lariat branchpoint sequences in one intron in a xeroderma pigmentosum DNA repair gene: mutations result in reduced XPC mRNA levels that correlate with cancer risk. *Hum. Mol. Genet.* 13, 343–352.
- Khan, S.G., Oh, K.-S., Emmert, S., Imoto, K., Tamura, D., Digiovanna, J.J., Shahlavi, T., Armstrong, N., Baker, C.C., Neuburg, M., et al. (2009). XPC initiation codon mutation in xeroderma pigmentosum patients with and without neurological symptoms. *DNA Repair* 8, 114–125.

- Kiel, M.J., Yilmaz, O.H., Iwashita, T., Yilmaz, O.H., Terhorst, C., and Morrison, S.J. (2005). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* *121*, 1109–1121.
- Kiel, M.J., He, S., Ashkenazi, R., Gentry, S.N., Teta, M., Kushner, J.A., Jackson, T.L., and Morrison, S.J. (2007). Haematopoietic stem cells do not asymmetrically segregate chromosomes or retain BrdU. *Nature* *449*, 238–242.
- Kiel, M.J., Yilmaz, O.H., and Morrison, S.J. (2008). CD150- cells are transiently reconstituting multipotent progenitors with little or no stem cell activity. *Blood* *111*, 4413–4414; author reply 4414–4415.
- Kim, J.K., Patel, D., and Choi, B.S. (1995). Contrasting structural impacts induced by cis-syn cyclobutane dimer and (6-4) adduct in DNA duplex decamers: implication in mutagenesis and repair activity. *Photochem. Photobiol.* *62*, 44–50.
- Kim, M., Moon, H.-B., and Spangrude, G.J. (2003). Major age-related changes of mouse hematopoietic stem/progenitor cells. *Ann. N. Y. Acad. Sci.* *996*, 195–208.
- Kim, M.J., Kim, M.H., Kim, S.A., and Chang, J.S. (2008). Age-related Deterioration of Hematopoietic Stem Cells. *Int. J. Stem Cells* 55–63.
- Koeffler, H.P., and Golde, D.W. (1978). Acute myelogenous leukemia: a human cell line responsive to colony-stimulating activity. *Science* *200*, 1153–1154.
- Koeffler, H.P., Billing, R., Lusic, A.J., Sparkes, R., and Golde, D.W. (1980). An undifferentiated variant derived from the human acute myelogenous leukemia cell line (KG-1). *Blood* *56*, 265–273.
- Koh, L.-P., and Chao, N.J. (2004). Umbilical cord blood transplantation in adults using myeloablative and nonmyeloablative preparative regimens. *Biol. Blood Marrow Transplant. J. Am. Soc. Blood Marrow Transplant.* *10*, 1–22.
- Kondo, M., Weissman, I.L., and Akashi, K. (1997). Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* *91*, 661–672.
- Kondo, M., Wagers, A.J., Manz, M.G., Prohaska, S.S., Scherer, D.C., Beilhack, G.F., Shizuru, J.A., and Weissman, I.L. (2003). Biology of hematopoietic stem cells and progenitors: implications for clinical application. *Annu. Rev. Immunol.* *21*, 759–806.
- Koptyra, M., Falinski, R., Nowicki, M.O., Stoklosa, T., Majsterek, I., Nieborowska-Skorska, M., Blasiak, J., and Skorski, T. (2006). BCR/ABL kinase induces self-mutagenesis via reactive oxygen species to encode imatinib resistance. *Blood* *108*, 319–327.
- Körbling, M., and Freireich, E.J. (2011). Twenty-five years of peripheral blood stem cell transplantation. *Blood* *117*, 6411–6416.
- Kraemer, K.H. (1997). Sunlight and skin cancer: Another link revealed. *Proc. Natl. Acad. Sci.* *94*, 11–14.
- Kraemer, K.H., Lee, M.M., and Scotto, J. (1984). DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. *Carcinogenesis* *5*, 511–514.
- Kraemer, K.H., Lee, M.M., and Scotto, J. (1987). Xeroderma pigmentosum. Cutaneous, ocular, and neurologic abnormalities in 830 published cases. *Arch. Dermatol.* *123*, 241–250.

- Kraemer, K.H., DiGiovanna, J.J., and Peck, G.L. (1992). Chemoprevention of skin cancer in xeroderma pigmentosum. *J. Dermatol.* *19*, 715–718.
- Krasikova, Y.S., Rechkunova, N.I., Maltseva, E.A., Petruseva, I.O., and Lavrik, O.I. (2010). Localization of xeroderma pigmentosum group A protein and replication protein A on damaged DNA in nucleotide excision repair. *Nucleic Acids Res.* *38*, 8083–8094.
- Krause, D.S., Theise, N.D., Collector, M.I., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S., and Sharkis, S.J. (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* *105*, 369–377.
- Kuranda, K., Vargaftig, J., de la Rochere, P., Dosquet, C., Charron, D., Bardin, F., Tonnel, C., Bonnet, D., and Goodhardt, M. (2011). Age-related changes in human hematopoietic stem/progenitor cells. *Aging Cell* *10*, 542–546.
- Kurimasa, A., Ouyang, H., Dong, L., Wang, S., Li, X., Cordon-Cardo, C., Chen, D.J., and Li, G.C. (1999). Catalytic subunit of DNA-dependent protein kinase: Impact on lymphocyte development and tumorigenesis. *Proc. Natl. Acad. Sci.* *96*, 1403–1408.
- Laat, W.L. de, Appeldoorn, E., Jaspers, N.G.J., and Hoeijmakers, J.H.J. (1998). DNA Structural Elements Required for ERCC1-XPF Endonuclease Activity. *J. Biol. Chem.* *273*, 7835–7842.
- De Laat, W.L., Jaspers, N.G., and Hoeijmakers, J.H. (1999). Molecular mechanism of nucleotide excision repair. *Genes Dev.* *13*, 768–785.
- Lafitte, M., Rousseau, B., Moranvillier, I., Taillepiere, M., Peuchant, E., Guyonnet-Dupérat, V., Bedel, A., Dubus, P., de Verneuil, H., Moreau-Gaudry, F., et al. (2012). In vivo gene transfer targeting in pancreatic adenocarcinoma with cell surface antigens. *Mol. Cancer* *11*, 81.
- Lagasse, E., Connors, H., Al-Dhalimy, M., Reitsma, M., Dohse, M., Osborne, L., Wang, X., Finegold, M., Weissman, I.L., and Grompe, M. (2000). Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat. Med.* *6*, 1229–1234.
- Langie, S.A.S., Knaapen, A.M., Houben, J.M.J., van Kempen, F.C., de Hoon, J.P.J., Gottschalk, R.W.H., Godschalk, R.W.L., and van Schooten, F.J. (2007). The role of glutathione in the regulation of nucleotide excision repair during oxidative stress. *Toxicol. Lett.* *168*, 302–309.
- Lansdorp, P.M., Dragowska, W., and Mayani, H. (1993). Ontogeny-related changes in proliferative potential of human hematopoietic cells. *J. Exp. Med.* *178*, 787–791.
- Laugel, V., Dalloz, C., Durand, M., Sauvanaud, F., Kristensen, U., Vincent, M.C., Pasquier, L., Odent, S., Cormier-Daire, V., Gener, B., et al. (2010). Mutation update for the CSB/ERCC6 and CSA/ERCC8 genes involved in Cockayne syndrome. *Hum. Mutat.* *31*, 113–126.
- De Laval, B., Pawlikowska, P., Petit-Cocault, L., Bilhou-Nabera, C., Aubin-Houzelstein, G., Souyri, M., Pouzoulet, F., Gaudry, M., and Porteu, F. (2013). Thrombopoietin-increased DNA-PK-dependent DNA repair limits hematopoietic stem and progenitor cell mutagenesis in response to DNA damage. *Cell Stem Cell* *12*, 37–48.
- Lee, K.-M., Choi, J.-Y., Kang, C., Kang, C.P., Park, S.K., Cho, H., Cho, D.-Y., Yoo, K.-Y., Noh, D.-Y., Ahn, S.-H., et al. (2005). Genetic polymorphisms of selected DNA repair genes,

- estrogen and progesterone receptor status, and breast cancer risk. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* *11*, 4620–4626.
- Legerski, R., and Peterson, C. (1992). Expression cloning of a human DNA repair gene involved in xeroderma pigmentosum group C. *Nature* *359*, 70–73.
- Li, X., and Heyer, W.-D. (2008). Homologous recombination in DNA repair and DNA damage tolerance. *Cell Res.* *18*, 99–113.
- Li, L., Bales, E.S., Peterson, C.A., and Legerski, R.J. (1993). Characterization of molecular defects in xeroderma pigmentosum group C. *Nat. Genet.* *5*, 413–417.
- Li, X., Erden, O., Li, L., Ye, Q., Wilson, A., and Du, W. (2014). Binding to WGR domain by salidroside activates PARP1 and protects hematopoietic stem cells from oxidative stress. *Antioxid. Redox Signal.* *20*, 1853–1865.
- Lieber, M.R. (2010). The Mechanism of Double-Strand DNA Break Repair by the Nonhomologous DNA End-Joining Pathway. *Annu. Rev. Biochem.* *79*, 181–211.
- Lieber, M.R., Lu, H., Gu, J., and Schwarz, K. (2008). Flexibility in the order of action and in the enzymology of the nuclease, polymerases, and ligase of vertebrate non-homologous DNA end joining: relevance to cancer, aging, and the immune system. *Cell Res.* *18*, 125–133.
- Lindahl, T. (1993). Instability and decay of the primary structure of DNA. *Nature* *362*, 709–715.
- Liu, S., Ginestier, C., Charafe-Jauffret, E., Foco, H., Kleer, C.G., Merajver, S.D., Dontu, G., and Wicha, M.S. (2008). BRCA1 regulates human mammary stem/progenitor cell fate. *Proc. Natl. Acad. Sci. U. S. A.* *105*, 1680–1685.
- Liu, S.-Y., Wen, C.-Y., Lee, Y.-J., and Lee, T.-C. (2010). XPC silencing sensitizes glioma cells to arsenic trioxide via increased oxidative damage. *Toxicol. Sci. Off. J. Soc. Toxicol.* *116*, 183–193.
- Lo, H.-L., Nakajima, S., Ma, L., Walter, B., Yasui, A., Ethell, D.W., and Owen, L.B. (2005). Differential biologic effects of CPD and 6-4PP UV-induced DNA damage on the induction of apoptosis and cell-cycle arrest. *BMC Cancer* *5*, 135.
- Loeffler, M., and Roeder, I. (2002). Tissue stem cells: definition, plasticity, heterogeneity, self-organization and models--a conceptual approach. *Cells Tissues Organs* *171*, 8–26.
- Lombard, D.B., Chua, K.F., Mostoslavsky, R., Franco, S., Gostissa, M., and Alt, F.W. (2005). DNA repair, genome stability, and aging. *Cell* *120*, 497–512.
- Lommel, L., Ortolan, T., Chen, L., Madura, K., and Sweder, K.S. (2002). Proteolysis of a nucleotide excision repair protein by the 26 S proteasome. *Curr. Genet.* *42*, 9–20.
- LORENZ, E., UPHOFF, D., REID, T.R., and SHELTON, E. (1951). Modification of irradiation injury in mice and guinea pigs by bone marrow injections. *J. Natl. Cancer Inst.* *12*, 197–201.
- Lu, M., Kawamoto, H., Katsube, Y., Ikawa, T., and Katsura, Y. (2002). The Common Myelolymphoid Progenitor: A Key Intermediate Stage in Hemopoiesis Generating T and B Cells. *J. Immunol.* *169*, 3519–3525.

- Lubin, A., Zhang, L., Chen, H., White, V.M., and Gong, F. (2014). A human XPC protein interactome--a resource. *Int. J. Mol. Sci.* *15*, 141–158.
- Ludin, A., Gur-Cohen, S., Golan, K., Kaufmann, K.B., Itkin, T., Medaglia, C., Lu, X.-J., Ledergor, G., Kollet, O., and Lapidot, T. (2014). Reactive Oxygen Species Regulate Hematopoietic Stem Cell Self-Renewal, Migration and Development, As Well As Their Bone Marrow Microenvironment. *Antioxid. Redox Signal.* *21*, 1605–1619.
- Luo, W., Hu, H., Chang, R., Zhong, J., Knabel, M., O’Meally, R., Cole, R.N., Pandey, A., and Semenza, G.L. (2011). Pyruvate Kinase M2 Is a PHD3-Stimulated Coactivator for Hypoxia-Inducible Factor 1. *Cell* *145*, 732–744.
- Månsson, R., Hultquist, A., Luc, S., Yang, L., Anderson, K., Kharazi, S., Al-Hashmi, S., Liuba, K., Thorén, L., Adolfsson, J., et al. (2007). Molecular Evidence for Hierarchical Transcriptional Lineage Priming in Fetal and Adult Stem Cells and Multipotent Progenitors. *Immunity* *26*, 407–419.
- Martin, G.R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. U. S. A.* *78*, 7634–7638.
- Matsuoka, S., Ballif, B.A., Smogorzewska, A., McDonald, E.R., 3rd, Hurov, K.E., Luo, J., Bakalarski, C.E., Zhao, Z., Solimini, N., Lerenthal, Y., et al. (2007). ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* *316*, 1160–1166.
- Mayle, A., Luo, M., Jeong, M., and Goodell, M.A. (2013). Flow cytometry analysis of murine hematopoietic stem cells. *Cytom. Part J. Int. Soc. Anal. Cytol.* *83*, 27–37.
- Le May, N., Mota-Fernandes, D., Vélez-Cruz, R., Iltis, I., Biard, D., and Egly, J.M. (2010). NER Factors Are Recruited to Active Promoters and Facilitate Chromatin Modification for Transcription in the Absence of Exogenous Genotoxic Attack. *Mol. Cell* *38*, 54–66.
- McIlwraith, M.J., Vaisman, A., Liu, Y., Fanning, E., Woodgate, R., and West, S.C. (2005). Human DNA Polymerase η Promotes DNA Synthesis from Strand Invasion Intermediates of Homologous Recombination. *Mol. Cell* *20*, 783–792.
- Mebius, R.E., Miyamoto, T., Christensen, J., Domen, J., Cupedo, T., Weissman, I.L., and Akashi, K. (2001). The Fetal Liver Counterpart of Adult Common Lymphoid Progenitors Gives Rise to All Lymphoid Lineages, CD45⁺CD4⁺CD3⁻ Cells, As Well As Macrophages. *J. Immunol.* *166*, 6593–6601.
- Meijne, E.I., van der Winden-van Groenewegen, R.J., Ploemacher, R.E., Vos, O., David, J.A., and Huiskamp, R. (1991). The effects of x-irradiation on hematopoietic stem cell compartments in the mouse. *Exp. Hematol.* *19*, 617–623.
- Meira, L.B., Reis, A.M., Cheo, D.L., Nahari, D., Burns, D.K., and Friedberg, E.C. (2001a). Cancer predisposition in mutant mice defective in multiple genetic pathways: uncovering important genetic interactions. *Mutat. Res.* *477*, 51–58.
- Meira, L.B., Reis, A.M.C., Cheo, D.L., Nahari, D., Burns, D.K., and Friedberg, E.C. (2001b). Cancer predisposition in mutant mice defective in multiple genetic pathways: uncovering important genetic interactions. *Mutat. Res. Mol. Mech. Mutagen.* *477*, 51–58.

- Melis, J.P.M., Wijnhoven, S.W.P., Beems, R.B., Roodbergen, M., van den Berg, J., Moon, H., Friedberg, E., van der Horst, G.T.J., Hoeijmakers, J.H.J., Vijg, J., et al. (2008a). Mouse Models for Xeroderma Pigmentosum Group A and Group C Show Divergent Cancer Phenotypes. *Cancer Res.* *68*, 1347–1353.
- Melis, J.P.M., Wijnhoven, S.W.P., Beems, R.B., Roodbergen, M., van den Berg, J., Moon, H., Friedberg, E., van der Horst, G.T.J., Hoeijmakers, J.H.J., Vijg, J., et al. (2008b). Mouse models for xeroderma pigmentosum group A and group C show divergent cancer phenotypes. *Cancer Res.* *68*, 1347–1353.
- Melis, J.P.M., Wijnhoven, S.W.P., Beems, R.B., Roodbergen, M., Berg, J. van den, Moon, H., Friedberg, E., Horst, G.T.J. van der, Hoeijmakers, J.H.J., Vijg, J., et al. (2008c). Mouse Models for Xeroderma Pigmentosum Group A and Group C Show Divergent Cancer Phenotypes. *Cancer Res.* *68*, 1347–1353.
- Melis, J.P.M., Luijten, M., Mullenders, L.H.F., and van Steeg, H. (2011). The role of XPC: implications in cancer and oxidative DNA damage. *Mutat. Res.* *728*, 107–117.
- Melis, J.P.M., van Steeg, H., and Luijten, M. (2013). Oxidative DNA damage and nucleotide excision repair. *Antioxid. Redox Signal.* *18*, 2409–2419.
- Melo, J.V., and Barnes, D.J. (2007). Chronic myeloid leukaemia as a model of disease evolution in human cancer. *Nat. Rev. Cancer* *7*, 441–453.
- Menck, C.F., and Munford, V. (2014). DNA repair diseases: What do they tell us about cancer and aging? *Genet. Mol. Biol.* *37*, 220–233.
- Menoni, H., Hoeijmakers, J.H.J., and Vermeulen, W. (2012). Nucleotide excision repair-initiating proteins bind to oxidative DNA lesions in vivo. *J. Cell Biol.* *199*, 1037–1046.
- Miao, F., Bouziane, M., Dammann, R., Masutani, C., Hanaoka, F., Pfeifer, G., and O'Connor, T.R. (2000). 3-Methyladenine-DNA glycosylase (MPG protein) interacts with human RAD23 proteins. *J. Biol. Chem.* *275*, 28433–28438.
- Miccoli, L., Burr, K.L.-A., Hickenbotham, P., Friedberg, E.C., Angulo, J.F., and Dubrova, Y.E. (2007). The combined effects of xeroderma pigmentosum C deficiency and mutagens on mutation rates in the mouse germ line. *Cancer Res.* *67*, 4695–4699.
- Mikkola, H.K.A., and Orkin, S.H. (2006). The journey of developing hematopoietic stem cells. *Dev. Camb. Engl.* *133*, 3733–3744.
- Milyavsky, M., Gan, O.I., Trottier, M., Komosa, M., Tabach, O., Notta, F., Lechman, E., Hermans, K.G., Eppert, K., Konovalova, Z., et al. (2010). A distinctive DNA damage response in human hematopoietic stem cells reveals an apoptosis-independent role for p53 in self-renewal. *Cell Stem Cell* *7*, 186–197.
- Min, J.-H., and Pavletich, N.P. (2007). Recognition of DNA damage by the Rad4 nucleotide excision repair protein. *Nature* *449*, 570–575.
- Mitalipov, S., and Wolf, D. (2009). Totipotency, pluripotency and nuclear reprogramming. *Adv. Biochem. Eng. Biotechnol.* *114*, 185–199.
- Mitchell, D.L., Haipek, C.A., and Clarkson, J.M. (1985). (6-4)Photoproducts are removed from the DNA of UV-irradiated mammalian cells more efficiently than cyclobutane pyrimidine dimers. *Mutat. Res.* *143*, 109–112.

- Modrich, P. (2006). Mechanisms in Eukaryotic Mismatch Repair. *J. Biol. Chem.* *281*, 30305–30309.
- Mohrin, M., Bourke, E., Alexander, D., Warr, M.R., Barry-Holson, K., Le Beau, M.M., Morrison, C.G., and Passegué, E. (2010). Hematopoietic stem cell quiescence promotes error-prone DNA repair and mutagenesis. *Cell Stem Cell* *7*, 174–185.
- Morita, Y., Ema, H., and Nakauchi, H. (2010). Heterogeneity and hierarchy within the most primitive hematopoietic stem cell compartment. *J. Exp. Med.* *207*, 1173–1182.
- Morrison, S.J., and Kimble, J. (2006). Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature* *441*, 1068–1074.
- Morrison, S.J., Shah, N.M., and Anderson, D.J. (1997). Regulatory mechanisms in stem cell biology. *Cell* *88*, 287–298.
- Mullaart, E., Lohman, P.H.M., Berends, F., and Vijg, J. (1990). DNA damage metabolism and aging. *Mutat. Res.* *237*, 189–210.
- Müller-Sieburg, C.E., Cho, R.H., Thoman, M., Adkins, B., and Sieburg, H.B. (2002). Deterministic regulation of hematopoietic stem cell self-renewal and differentiation. *Blood* *100*, 1302–1309.
- Muller-Sieburg, C.E., Cho, R.H., Karlsson, L., Huang, J.-F., and Sieburg, H.B. (2004). Myeloid-biased hematopoietic stem cells have extensive self-renewal capacity but generate diminished lymphoid progeny with impaired IL-7 responsiveness. *Blood* *103*, 4111–4118.
- Nakatsu, Y., Asahina, H., Citterio, E., Rademarkers, S., Vermeulen, W., Kamiuchi, S., Yeo, J.-P., Khaw, M.-C., Saijo, M., Kodo, N., et al. (2000). XAB2, a novel tetratricopeptide repeat protein, involved in transcription-coupled DNA repair and transcription. *J. Biol. Chem.*
- Nance, M.A., and Berry, S.A. (1992). Cockayne syndrome: review of 140 cases. *Am. J. Med. Genet.* *42*, 68–84.
- Navarrete, C., and Contreras, M. (2009). Cord blood banking: a historical perspective. *Br. J. Haematol.* *147*, 236–245.
- Navarro, S., Meza, N.W., Quintana-Bustamante, O., Casado, J.A., Jacome, A., McAllister, K., Puerto, S., Surrallés, J., Segovia, J.C., and Bueren, J.A. (2006). Hematopoietic dysfunction in a mouse model for Fanconi anemia group D1. *Mol. Ther. J. Am. Soc. Gene Ther.* *14*, 525–535.
- Neumann, A.S., Sturgis, E.M., and Wei, Q. (2005). Nucleotide excision repair as a marker for susceptibility to tobacco-related cancers: a review of molecular epidemiological studies. *Mol. Carcinog.* *42*, 65–92.
- Ng, J.M.Y., Vermeulen, W., van der Horst, G.T.J., Bergink, S., Sugawara, K., Vrieling, H., and Hoeijmakers, J.H.J. (2003). A novel regulation mechanism of DNA repair by damage-induced and RAD23-dependent stabilization of xeroderma pigmentosum group C protein. *Genes Dev.* *17*, 1630–1645.
- Nguyen, T.-A., Slattery, S.D., Moon, S.-H., Darlington, Y.F., Lu, X., and Donehower, L.A. (2010). The oncogenic phosphatase WIP1 negatively regulates nucleotide excision repair. *DNA Repair* *9*, 813–823.

- Niedernhofer, L.J. (2008a). DNA repair is crucial for maintaining hematopoietic stem cell function. *DNA Repair* 7, 523–529.
- Niedernhofer, L.J. (2008b). DNA repair is crucial for maintaining hematopoietic stem cell function. *DNA Repair* 7, 523–529.
- Nijnik, A., Woodbine, L., Marchetti, C., Dawson, S., Lambe, T., Liu, C., Rodrigues, N.P., Crockford, T.L., Cabuy, E., Vindigni, A., et al. (2007). DNA repair is limiting for haematopoietic stem cells during ageing. *Nature* 447, 686–690.
- Nimonkar, A.V., Özsoy, A.Z., Genschel, J., Modrich, P., and Kowalczykowski, S.C. (2008). Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. *Proc. Natl. Acad. Sci.* 105, 16906–16911.
- Nishi, R., Okuda, Y., Watanabe, E., Mori, T., Iwai, S., Masutani, C., Sugasawa, K., and Hanaoka, F. (2005). Centrin 2 stimulates nucleotide excision repair by interacting with xeroderma pigmentosum group C protein. *Mol. Cell. Biol.* 25, 5664–5674.
- Nitta, E., Yamashita, M., Hosokawa, K., Xian, M., Takubo, K., Arai, F., Nakada, S., and Suda, T. (2011). Telomerase reverse transcriptase protects ATM-deficient hematopoietic stem cells from ROS-induced apoptosis through a telomere-independent mechanism. *Blood* 117, 4169–4180.
- Noble, M., Mayer-Pröschel, M., and Pröschel, C. (2005). Redox Regulation of Precursor Cell Function: Insights and Paradoxes. *Antioxid. Redox Signal.* 7, 1456–1467.
- Nohl, H., Gille, L., and Staniek, K. (2005). Intracellular generation of reactive oxygen species by mitochondria. *Biochem. Pharmacol.* 69, 719–723.
- Notta, F., Doulatov, S., Laurenti, E., Poeppl, A., Jurisica, I., and Dick, J.E. (2011). Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science* 333, 218–221.
- Nospikel, T. (2007). DNA repair in differentiated cells: some new answers to old questions. *Neuroscience* 145, 1213–1221.
- Nospikel, T. (2011). Multiple roles of ubiquitination in the control of nucleotide excision repair. *Mech. Ageing Dev.* 132, 355–365.
- Nospikel, T., and Hanawalt, P.C. (2000a). Terminally differentiated human neurons repair transcribed genes but display attenuated global DNA repair and modulation of repair gene expression. *Mol. Cell. Biol.* 20, 1562–1570.
- Nospikel, T., and Hanawalt, P.C. (2000b). Terminally differentiated human neurons repair transcribed genes but display attenuated global DNA repair and modulation of repair gene expression. *Mol. Cell. Biol.* 20, 1562–1570.
- Nospikel, T., and Hanawalt, P.C. (2002). DNA repair in terminally differentiated cells. *DNA Repair* 1, 59–75.
- Oguro, H., Ding, L., and Morrison, S.J. (2013). SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. *Cell Stem Cell* 13, 102–116.

Okamoto, Y., Chou, P.-H., Kim, S.Y., Suzuki, N., Laxmi, Y.R.S., Okamoto, K., Liu, X., Matsuda, T., and Shibutani, S. (2008). Oxidative DNA damage in XPC-knockout and its wild mice treated with equine estrogen. *Chem. Res. Toxicol.* *21*, 1120–1124.

Orford, K.W., and Scadden, D.T. (2008). Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation. *Nat. Rev. Genet.* *9*, 115–128.

Orkin, S.H., and Zon, L.I. (2008). Hematopoiesis: an evolving paradigm for stem cell biology. *Cell* *132*, 631–644.

Osawa, M., Hanada, K., Hamada, H., and Nakauchi, H. (1996). Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* *273*, 242–245.

Pang, W.W., Price, E.A., Sahoo, D., Beerman, I., Maloney, W.J., Rossi, D.J., Schrier, S.L., and Weissman, I.L. (2011). Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. *Proc. Natl. Acad. Sci. U. S. A.* *108*, 20012–20017.

Park, C.-J., and Choi, B.-S. (2006). The protein shuffle. Sequential interactions among components of the human nucleotide excision repair pathway. *FEBS J.* *273*, 1600–1608.

Park, H., Zhang, K., Ren, Y., Nadji, S., Sinha, N., Taylor, J.-S., and Kang, C. (2002). Crystal structure of a DNA decamer containing a cis-syn thymine dimer. *Proc. Natl. Acad. Sci. U. S. A.* *99*, 15965–15970.

Parmar, K., Kim, J., Sykes, S.M., Shimamura, A., Stuckert, P., Zhu, K., Hamilton, A., Deloach, M.K., Kutok, J.L., Akashi, K., et al. (2010). Hematopoietic stem cell defects in mice with deficiency of *Fancd2* or *Usp1*. *Stem Cells Dayt. Ohio* *28*, 1186–1195.

Passegué, E. (2005). Hematopoietic stem cells, leukemic stem cells and chronic myelogenous leukemia. *Cell Cycle Georget. Tex* *4*, 266–268.

Passegué, E., Wagers, A.J., Giuriato, S., Anderson, W.C., and Weissman, I.L. (2005). Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. *J. Exp. Med.* *202*, 1599–1611.

Perrotti, D., Jamieson, C., Goldman, J., and Skorski, T. (2010). Chronic myeloid leukemia: mechanisms of blastic transformation. *J. Clin. Invest.* *120*, 2254–2264.

Piccoli, C., Ria, R., Scrima, R., Cela, O., D'Aprile, A., Boffoli, D., Falzetti, F., Tabilio, A., and Capitanio, N. (2005). Characterization of mitochondrial and extra-mitochondrial oxygen consuming reactions in human hematopoietic stem cells. Novel evidence of the occurrence of NAD(P)H oxidase activity. *J. Biol. Chem.* *280*, 26467–26476.

Piccoli, C., D'Aprile, A., Ripoli, M., Scrima, R., Lecce, L., Boffoli, D., Tabilio, A., and Capitanio, N. (2007). Bone-marrow derived hematopoietic stem/progenitor cells express multiple isoforms of NADPH oxidase and produce constitutively reactive oxygen species. *Biochem. Biophys. Res. Commun.* *353*, 965–972.

Ploemacher, R.E., and Brons, N.H. (1988). Cells with marrow and spleen repopulating ability and forming spleen colonies on day 16, 12, and 8 are sequentially ordered on the basis of increasing rhodamine 123 retention. *J. Cell. Physiol.* *136*, 531–536.

Porto, L., Weis, R., Schulz, C., Reichel, P., Lanfermann, H., and Zanella, F.E. (2000). Tay's syndrome: MRI. *Neuroradiology* *42*, 849–851.

- Poulsen, S.L., Hansen, R.K., Wagner, S.A., van Cuijk, L., van Belle, G.J., Streicher, W., Wikström, M., Choudhary, C., Houtsmuller, A.B., Marteijn, J.A., et al. (2013). RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. *J. Cell Biol.* *201*, 797–807.
- Prall, W.C., Czibere, A., Jäger, M., Spentzos, D., Libermann, T.A., Gattermann, N., Haas, R., and Aivado, M. (2007). Age-related transcription levels of KU70, MGST1 and BIK in CD34+ hematopoietic stem and progenitor cells. *Mech. Ageing Dev.* *128*, 503–510.
- Prasher, J.M., Lalai, A.S., Heijmans-Antonissen, C., Ploemacher, R.E., Hoeijmakers, J.H.J., Touw, I.P., and Niedernhofer, L.J. (2005). Reduced hematopoietic reserves in DNA interstrand crosslink repair-deficient *Ercc1*^{-/-} mice. *EMBO J.* *24*, 861–871.
- Priestley, G.V., Scott, L.M., Ulyanova, T., and Papayannopoulou, T. (2006). Lack of alpha4 integrin expression in stem cells restricts competitive function and self-renewal activity. *Blood* *107*, 2959–2967.
- Qing, Y., Wang, Z., Bunting, K.D., and Gerson, S.L. (2014). Bcl2 overexpression rescues the hematopoietic stem cell defects in Ku70-deficient mice by restoration of quiescence. *Blood* *123*, 1002–1011.
- Quéré, R., Saint-Paul, L., Carmignac, V., Martin, R.Z., Chrétien, M.-L., Largeot, A., Hammann, A., Pais de Barros, J.-P., Bastie, J.-N., and Delva, L. (2014). Tif1 γ regulates the TGF- β 1 receptor and promotes physiological aging of hematopoietic stem cells. *Proc. Natl. Acad. Sci. U. S. A.* *111*, 10592–10597.
- Quinn, M.T., and Gauss, K.A. (2004). Structure and regulation of the neutrophil respiratory burst oxidase: comparison with nonphagocyte oxidases. *J. Leukoc. Biol.* *76*, 760–781.
- Raaijmakers, M.H.G.P., Mukherjee, S., Guo, S., Zhang, S., Kobayashi, T., Schoonmaker, J.A., Ebert, B.L., Al-Shahrour, F., Hasserjian, R.P., Scadden, E.O., et al. (2010). Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature* *464*, 852–857.
- Ratajczak, M. (2008). Phenotypic and functional characterization of hematopoietic stem cells. [Miscellaneous Article]. *Curr. Opin. Hematol.* July 2008 *15*, 293–300.
- Reddy, N.P., Vemuri, M.C., and Pallu, R. (2007). Isolation of stem cells from human umbilical cord blood. *Methods Mol. Biol. Clifton NJ* *407*, 149–163.
- Reese, J.S., Liu, L., and Gerson, S.L. (2003). Repopulating defect of mismatch repair-deficient hematopoietic stem cells. *Blood* *102*, 1626–1633.
- Reya, T., Morrison, S.J., Clarke, M.F., and Weissman, I.L. (2001). Stem cells, cancer, and cancer stem cells. *Nature* *414*, 105–111.
- Rezvani, H.R., Dedieu, S., North, S., Belloc, F., Rossignol, R., Letellier, T., de Verneuil, H., Taïeb, A., and Mazurier, F. (2007). Hypoxia-inducible factor-1alpha, a key factor in the keratinocyte response to UVB exposure. *J. Biol. Chem.* *282*, 16413–16422.
- Rezvani, H.R., Mahfouf, W., Ali, N., Chemin, C., Ged, C., Kim, A.L., de Verneuil, H., Taïeb, A., Bickers, D.R., and Mazurier, F. (2010). Hypoxia-inducible factor-1alpha regulates the expression of nucleotide excision repair proteins in keratinocytes. *Nucleic Acids Res.* *38*, 797–809.

Rezvani, H.R., Rossignol, R., Ali, N., Benard, G., Tang, X., Yang, H.S., Jouary, T., de Verneuil, H., Taïeb, A., Kim, A.L., et al. (2011a). XPC silencing in normal human keratinocytes triggers metabolic alterations through NOX-1 activation-mediated reactive oxygen species. *Biochim. Biophys. Acta* 1807, 609–619.

Rezvani, H.R., Kim, A.L., Rossignol, R., Ali, N., Daly, M., Mahfouf, W., Bellance, N., Taïeb, A., de Verneuil, H., Mazurier, F., et al. (2011b). XPC silencing in normal human keratinocytes triggers metabolic alterations that drive the formation of squamous cell carcinomas. *J. Clin. Invest.* 121, 195–211.

Rezvani, H.R., Rossignol, R., Ali, N., Benard, G., Tang, X., Yang, H.S., Jouary, T., de Verneuil, H., Taïeb, A., Kim, A.L., et al. (2011c). XPC silencing in normal human keratinocytes triggers metabolic alterations through NOX-1 activation-mediated reactive oxygen species. *Biochim. Biophys. Acta* 1807, 609–619.

Robert-Richard, E., Ged, C., Ortet, J., Santarelli, X., Lamrissi-Garcia, I., de Verneuil, H., and Mazurier, F. (2006). Human cell engraftment after busulfan or irradiation conditioning of NOD/SCID mice. *Haematologica* 91, 1384.

Robert-Richard, E., Lalanne, M., Lamrissi-Garcia, I., Guyonnet-Duperat, V., Richard, E., Pitard, V., Mazurier, F., Moreau-Gaudry, F., Ged, C., and de Verneuil, H. (2010). Modeling of congenital erythropoietic porphyria by RNA interference: a new tool for preclinical gene therapy evaluation. *J. Gene Med.* 12, 637–646.

Romanenko, A., Morimura, K., Wanibuchi, H., Salim, E.I., Kinoshita, A., Kaneko, M., Voizianov, A., and Fukushima, S. (2000). Increased oxidative stress with gene alteration in urinary bladder urothelium after the Chernobyl accident. *Int. J. Cancer* 86, 790–798.

Rossi, D.J., Bryder, D., Zahn, J.M., Ahlenius, H., Sonu, R., Wagers, A.J., and Weissman, I.L. (2005). Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc. Natl. Acad. Sci. U. S. A.* 102, 9194–9199.

Rossi, D.J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J., and Weissman, I.L. (2007a). Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* 447, 725–729.

Rossi, D.J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J., and Weissman, I.L. (2007b). Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* 447, 725–729.

Rossi, D.J., Jamieson, C.H.M., and Weissman, I.L. (2008). Stems cells and the pathways to aging and cancer. *Cell* 132, 681–696.

Rovera, G., Santoli, D., and Damsky, C. (1979). Human promyelocytic leukemia cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. *Proc. Natl. Acad. Sci. U. S. A.* 76, 2779–2783.

Rübe, C.E., Fricke, A., Widmann, T.A., Fürst, T., Madry, H., Pfreundschuh, M., and Rübe, C. (2011). Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging. *PloS One* 6, e17487.

Ruzankina, Y., Pinzon-Guzman, C., Asare, A., Ong, T., Pontano, L., Cotsarelis, G., Zediak, V.P., Velez, M., Bhandoola, A., and Brown, E.J. (2007). Deletion of the developmentally

essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss. *Cell Stem Cell* 1, 113–126.

Salim, E.I., Morimura, K., Menesi, A., El-Lity, M., Fukushima, S., and Wanibuchi, H. (2008). Elevated oxidative stress and DNA damage and repair levels in urinary bladder carcinomas associated with schistosomiasis. *Int. J. Cancer* 123, 601–608.

Sallmyr, A., Fan, J., and Rassool, F.V. (2008a). Genomic instability in myeloid malignancies: increased reactive oxygen species (ROS), DNA double strand breaks (DSBs) and error-prone repair. *Cancer Lett.* 270, 1–9.

Sallmyr, A., Tomkinson, A.E., and Rassool, F.V. (2008b). Up-regulation of WRN and DNA ligase III α in chronic myeloid leukemia: consequences for the repair of DNA double-strand breaks. *Blood* 112, 1413–1423.

Salob, S.P., Webb, D.K., and Atherton, D.J. (1992). A child with xeroderma pigmentosum and bone marrow failure. *Br. J. Dermatol.* 126, 372–374.

Sancar, A. (1996). DNA excision repair. *Annu. Rev. Biochem.* 65, 43–81.

Sancar, A., Lindsey-Boltz, L.A., Unsal-Kaçmaz, K., and Linn, S. (2004). Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu. Rev. Biochem.* 73, 39–85.

Sands, A.T., Abuin, A., Sanchez, A., Conti, C.J., and Bradley, A. (1995). High susceptibility to ultraviolet-induced carcinogenesis in mice lacking XPC. *Nature* 377, 162–165.

Santos, L.S., Gomes, B.C., Gouveia, R., Silva, S.N., Azevedo, A.P., Camacho, V., Manita, I., Gil, O.M., Ferreira, T.C., Limbert, E., et al. (2013). The role of CCNH Val270Ala (rs2230641) and other nucleotide excision repair polymorphisms in individual susceptibility to well-differentiated thyroid cancer. *Oncol. Rep.* 30, 2458–2466.

Sartori, A.A., Lukas, C., Coates, J., Mistrik, M., Fu, S., Bartek, J., Baer, R., Lukas, J., and Jackson, S.P. (2007). Human CtIP promotes DNA end resection. *Nature* 450, 509–514.

Sauer, H., Wartenberg, M., and Hescheler, J. (2001). Reactive Oxygen Species as Intracellular Messengers During Cell Growth and Differentiation. *Cell. Physiol. Biochem.* 11, 173–186.

Schlessinger, D., and Van Zant, G. (2001). Does functional depletion of stem cells drive aging? *Mech. Ageing Dev.* 122, 1537–1553.

Schofield, R. (1978). The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* 4, 7–25.

Seita, J., Rossi, D.J., and Weissman, I.L. (2010). Differential DNA damage response in stem and progenitor cells. *Cell Stem Cell* 7, 145–147.

Seki, M., Nakagawa, T., Seki, T., Kato, G., Tada, S., Takahashi, Y., Yoshimura, A., Kobayashi, T., Aoki, A., Otsuki, M., et al. (2006). Bloom Helicase and DNA Topoisomerase III α Are Involved in the Dissolution of Sister Chromatids. *Mol. Cell. Biol.* 26, 6299–6307.

Semenza, G.L. (2009). Regulation of cancer cell metabolism by hypoxia-inducible factor 1. *Semin. Cancer Biol.* 19, 12–16.

- Semenza, G.L. (2010). Oxygen homeostasis. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 2, 336–361.
- Seril, D.N., Liao, J., Yang, G.-Y., and Yang, C.S. (2003). Oxidative stress and ulcerative colitis-associated carcinogenesis: studies in humans and animal models. *Carcinogenesis* 24, 353–362.
- Shao, L., Feng, W., Lee, K.-J., Chen, B.P.C., and Zhou, D. (2012). A Sensitive and Quantitative Polymerase Chain Reaction-Based Cell Free In Vitro Non-Homologous End Joining Assay for Hematopoietic Stem Cells. *PLoS ONE* 7, e33499.
- Shepherd, B.E., Gutterop, P., Lansdorp, P.M., and Abkowitz, J.L. (2004). Estimating human hematopoietic stem cell kinetics using granulocyte telomere lengths. *Exp. Hematol.* 32, 1040–1050.
- Shiloh, Y. (2003). ATM and related protein kinases: safeguarding genome integrity. *Nat. Rev. Cancer* 3, 155–168.
- Shima, H., Takubo, K., Tago, N., Iwasaki, H., Arai, F., Takahashi, T., and Suda, T. (2010). Acquisition of G0 state by CD34-positive cord blood cells after bone marrow transplantation. *Exp. Hematol.* 38, 1231–1240.
- Shimizu, Y., Iwai, S., Hanaoka, F., and Sugasawa, K. (2003a). Xeroderma pigmentosum group C protein interacts physically and functionally with thymine DNA glycosylase. *EMBO J.* 22, 164–173.
- Shimizu, Y., Iwai, S., Hanaoka, F., and Sugasawa, K. (2003b). Xeroderma pigmentosum group C protein interacts physically and functionally with thymine DNA glycosylase. *EMBO J.* 22, 164–173.
- Shivji, M.K.K., Moggs, J.G., Kuraoka, I., and Wood, R.D. (2006). Assaying for the dual incisions of nucleotide excision repair using DNA with a lesion at a specific site. *Methods Mol. Biol. Clifton NJ* 314, 435–456.
- Da Silva, C.L., Gonçalves, R., dos Santos, F., Andrade, P.Z., Almeida-Porada, G., and Cabral, J.M.S. (2010). Dynamic cell-cell interactions between cord blood haematopoietic progenitors and the cellular niche are essential for the expansion of CD34+, CD34+CD38- and early lymphoid CD7+ cells. *J. Tissue Eng. Regen. Med.* 4, 149–158.
- SIMINOVITCH, L., MCCULLOCH, E.A., and TILL, J.E. (1963). THE DISTRIBUTION OF COLONY-FORMING CELLS AMONG SPLEEN COLONIES. *J. Cell. Physiol.* 62, 327–336.
- Simonnet, A.J., Nehmé, J., Vaigot, P., Barroca, V., Leboulch, P., and Tronik-Le Roux, D. (2009). Phenotypic and functional changes induced in hematopoietic stem/progenitor cells after gamma-ray radiation exposure. *Stem Cells Dayt. Ohio* 27, 1400–1409.
- Simsek, T., Kocabas, F., Zheng, J., DeBerardinis, R.J., Mahmoud, A.I., Olson, E.N., Schneider, J.W., Zhang, C.C., and Sadek, H.A. (2010). The Distinct Metabolic Profile of Hematopoietic Stem Cells Reflects Their Location in a Hypoxic Niche. *Cell Stem Cell* 7, 380–390.
- Sliwinski, T., Czechowska, A., Szemraj, J., Morawiec, Z., Skorski, T., and Blasiak, J. (2008). STI571 reduces NER activity in BCR/ABL-expressing cells. *Mutat. Res.* 654, 162–167.

- Slupianek, A., Hoser, G., Majsterek, I., Bronisz, A., Malecki, M., Blasiak, J., Fishel, R., and Skorski, T. (2002). Fusion tyrosine kinases induce drug resistance by stimulation of homology-dependent recombination repair, prolongation of G(2)/M phase, and protection from apoptosis. *Mol. Cell. Biol.* *22*, 4189–4201.
- Slupianek, A., Nowicki, M.O., Koptyra, M., and Skorski, T. (2006). BCR/ABL modifies the kinetics and fidelity of DNA double-strand breaks repair in hematopoietic cells. *DNA Repair* *5*, 243–250.
- Smith, C. (2003). Hematopoietic stem cells and hematopoiesis. *Cancer Control J. Moffitt Cancer Cent.* *10*, 9–16.
- Soini, Y., Haapasaari, K.-M., Vaarala, M.H., Turpeenniemi-Hujanen, T., Kärjä, V., and Karihtala, P. (2011). 8-hydroxydeguanosine and nitrotyrosine are prognostic factors in urinary bladder carcinoma. *Int. J. Clin. Exp. Pathol.* *4*, 267–275.
- Soto-Gutierrez, A., Navarro-Alvarez, N., Yagi, H., and Yarmush, M.L. (2009). Stem cells for liver repopulation. *Curr. Opin. Organ Transplant.* *14*, 667–673.
- Soufir, N., Ged, C., Bourillon, A., Austerlitz, F., Chemin, C., Stary, A., Armier, J., Pham, D., Khadir, K., Roume, J., et al. (2010). A prevalent mutation with founder effect in xeroderma pigmentosum group C from north Africa. *J. Invest. Dermatol.* *130*, 1537–1542.
- Spangrude, G.J., and Johnson, G.R. (1990). Resting and activated subsets of mouse multipotent hematopoietic stem cells. *Proc. Natl. Acad. Sci. U. S. A.* *87*, 7433–7437.
- Spangrude, G.J., Heimfeld, S., and Weissman, I.L. (1988). Purification and characterization of mouse hematopoietic stem cells. *Science* *241*, 58–62.
- Staresinic, L., Fagbemi, A.F., Enzlin, J.H., Gourdin, A.M., Wijgers, N., Dunand-Sauthier, I., Giglia-Mari, G., Clarkson, S.G., Vermeulen, W., and Schärer, O.D. (2009). Coordination of dual incision and repair synthesis in human nucleotide excision repair. *EMBO J.* *28*, 1111–1120.
- Stoklosa, T., Poplawski, T., Koptyra, M., Nieborowska-Skorska, M., Basak, G., Slupianek, A., Rayevskaya, M., Seferynska, I., Herrera, L., Blasiak, J., et al. (2008). BCR/ABL inhibits mismatch repair to protect from apoptosis and induce point mutations. *Cancer Res.* *68*, 2576–2580.
- Stout, G.J., Oosten, M. van, Acherrat, F.Z., Wit, J. de, Vermeij, W.P., Mullenders, L.H.F., Gruijl, F.R. de, and Backendorf, C. (2005). Selective DNA damage responses in murine Xpa^{-/-}, Xpc^{-/-} and Csb^{-/-} keratinocyte cultures. *DNA Repair* *4*, 1337–1344.
- Strom, S.S., Estey, E., Outschoorn, U.M., and Garcia-Manero, G. (2010). Acute myeloid leukemia outcome: role of nucleotide excision repair polymorphisms in intermediate risk patients. *Leuk. Lymphoma* *51*, 598–605.
- Suda, T., Suda, J., and Ogawa, M. (1984). Disparate differentiation in mouse hemopoietic colonies derived from paired progenitors. *Proc. Natl. Acad. Sci.* *81*, 2520–2524.
- Suda, T., Takubo, K., and Semenza, G.L. (2011). Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell* *9*, 298–310.
- Sugasawa, K. (2006a). UV-induced ubiquitylation of XPC complex, the UV-DDB-ubiquitin ligase complex, and DNA repair. *J. Mol. Histol.* *37*, 189–202.

- Sugasawa, K. (2006b). UV-induced ubiquitylation of XPC complex, the UV-DDB-ubiquitin ligase complex, and DNA repair. *J. Mol. Histol.* *37*, 189–202.
- Sugasawa, K., Okamoto, T., Shimizu, Y., Masutani, C., Iwai, S., and Hanaoka, F. (2001). A multistep damage recognition mechanism for global genomic nucleotide excision repair. *Genes Dev.* *15*, 507–521.
- Sugasawa, K., Shimizu, Y., Iwai, S., and Hanaoka, F. (2002). A molecular mechanism for DNA damage recognition by the xeroderma pigmentosum group C protein complex. *DNA Repair* *1*, 95–107.
- Sugasawa, K., Okuda, Y., Saijo, M., Nishi, R., Matsuda, N., Chu, G., Mori, T., Iwai, S., Tanaka, K., Tanaka, K., et al. (2005). UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. *Cell* *121*, 387–400.
- Sung, H.J., Ma, W., Starost, M.F., Lago, C.U., Lim, P.K., Sack, M.N., Kang, J.-G., Wang, P., and Hwang, P.M. (2011). Ambient oxygen promotes tumorigenesis. *PloS One* *6*, e19785.
- Szilvassy, S.J., and Cory, S. (1993). Phenotypic and functional characterization of competitive long-term repopulating hematopoietic stem cells enriched from 5-fluorouracil-treated murine marrow. *Blood* *81*, 2310–2320.
- Takano, H., Ema, H., Sudo, K., and Nakauchi, H. (2004). Asymmetric Division and Lineage Commitment at the Level of Hematopoietic Stem Cells Inference from Differentiation in Daughter Cell and Granddaughter Cell Pairs. *J. Exp. Med.* *199*, 295–302.
- Takizawa, H., Regoes, R.R., Boddupalli, C.S., Bonhoeffer, S., and Manz, M.G. (2011). Dynamic variation in cycling of hematopoietic stem cells in steady state and inflammation. *J. Exp. Med.* *208*, 273–284.
- Takubo, K., Ohmura, M., Azuma, M., Nagamatsu, G., Yamada, W., Arai, F., Hirao, A., and Suda, T. (2008). Stem cell defects in ATM-deficient undifferentiated spermatogonia through DNA damage-induced cell-cycle arrest. *Cell Stem Cell* *2*, 170–182.
- Takubo, K., Goda, N., Yamada, W., Iriuchishima, H., Ikeda, E., Kubota, Y., Shima, H., Johnson, R.S., Hirao, A., Suematsu, M., et al. (2010). Regulation of the HIF-1 α Level Is Essential for Hematopoietic Stem Cells. *Cell Stem Cell* *7*, 391–402.
- Tallman, M.S., Gilliland, D.G., and Rowe, J.M. (2005). Drug therapy for acute myeloid leukemia. *Blood* *106*, 1154–1163.
- Tang, J.Y., Hwang, B.J., Ford, J.M., Hanawalt, P.C., and Chu, G. (2000). Xeroderma pigmentosum p48 gene enhances global genomic repair and suppresses UV-induced mutagenesis. *Mol. Cell* *5*, 737–744.
- Tantin, D. (1998). RNA polymerase II elongation complexes containing the Cockayne syndrome group B protein interact with a molecular complex containing the transcription factor IIIH components xeroderma pigmentosum B and p62. *J. Biol. Chem.* *273*, 27794–27799.
- Tantin, D., Kansal, A., and Carey, M. (1997). Recruitment of the putative transcription-repair coupling factor CSB/ERCC6 to RNA polymerase II elongation complexes. *Mol. Cell. Biol.* *17*, 6803–6814.

- Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science* 282, 1145–1147.
- Till, J.E., and McCulloch, E.A. (1961). A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells. *Radiat. Res.* 14, 213–222.
- Tothova, Z., and Gilliland, D.G. (2007). FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. *Cell Stem Cell* 1, 140–152.
- Tran, P.T., Erdeniz, N., Symington, L.S., and Liskay, R.M. (2004). EXO1-A multi-tasking eukaryotic nuclease. *DNA Repair* 3, 1549–1559.
- Traver, D., Miyamoto, T., Christensen, J., Iwasaki-Arai, J., Akashi, K., and Weissman, I.L. (2001). Fetal liver myelopoiesis occurs through distinct, prospectively isolatable progenitor subsets. *Blood* 98, 627–635.
- Trego, K.S., and Turchi, J.J. (2006). Pre-steady-state binding of damaged DNA by XPC-hHR23B reveals a kinetic mechanism for damage discrimination. *Biochemistry (Mosc.)* 45, 1961–1969.
- Urnov, F.D., Rebar, E.J., Holmes, M.C., Zhang, H.S., and Gregory, P.D. (2010). Genome editing with engineered zinc finger nucleases. *Nat. Rev. Genet.* 11, 636–646.
- Ushio-Fukai, M., and Rehman, J. (2014). Redox and Metabolic Regulation of Stem/Progenitor Cells and Their Niche. *Antioxid. Redox Signal.* 21, 1587–1590.
- Varnum-Finney, B., Wu, L., Yu, M., Brashem-Stein, C., Staats, S., Flowers, D., Griffin, J.D., and Bernstein, I.D. (2000). Immobilization of Notch ligand, Delta-1, is required for induction of notch signaling. *J. Cell Sci.* 113 Pt 23, 4313–4318.
- Vaziri, H., Dragowska, W., Allsopp, R.C., Thomas, T.E., Harley, C.B., and Lansdorp, P.M. (1994). Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc. Natl. Acad. Sci. U. S. A.* 91, 9857–9860.
- Viale, A., De Franco, F., Orleth, A., Cambiaghi, V., Giuliani, V., Bossi, D., Ronchini, C., Ronzoni, S., Muradore, I., Monestiroli, S., et al. (2009). Cell-cycle restriction limits DNA damage and maintains self-renewal of leukaemia stem cells. *Nature* 457, 51–56.
- Villa, A., Snyder, E.Y., Vescovi, A., and Martínez-Serrano, A. (2000). Establishment and properties of a growth factor-dependent, perpetual neural stem cell line from the human CNS. *Exp. Neurol.* 161, 67–84.
- Walker, J.R., Corpina, R.A., and Goldberg, J. (2001). Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* 412, 607–614.
- Wang, G.L., and Semenza, G.L. (1995). Purification and characterization of hypoxia-inducible factor 1. *J. Biol. Chem.* 270, 1230–1237.
- Wang, G., Chuang, L., Zhang, X., Colton, S., Dombkowski, A., Reiners, J., Diakiw, A., and Xu, X.S. (2004). The initiative role of XPC protein in cisplatin DNA damaging treatment-mediated cell cycle regulation. *Nucleic Acids Res.* 32, 2231–2240.

- Wang, J., Cao, H., You, C., Yuan, B., Bahde, R., Gupta, S., Nishigori, C., Niedernhofer, L.J., Brooks, P.J., and Wang, Y. (2012). Endogenous formation and repair of oxidatively induced G[8-5 m]T intrastrand cross-link lesion. *Nucleic Acids Res.* *40*, 7368–7374.
- Wang, Q.-E., Zhu, Q., Wani, G., El-Mahdy, M.A., Li, J., and Wani, A.A. (2005). DNA repair factor XPC is modified by SUMO-1 and ubiquitin following UV irradiation. *Nucleic Acids Res.* *33*, 4023–4034.
- Wang, Q.-E., Praetorius-Ibba, M., Zhu, Q., El-Mahdy, M.A., Wani, G., Zhao, Q., Qin, S., Patnaik, S., and Wani, A.A. (2007). Ubiquitylation-independent degradation of Xeroderma pigmentosum group C protein is required for efficient nucleotide excision repair. *Nucleic Acids Res.* *35*, 5338–5350.
- Wang, Y., Liu, L., Pazhanisamy, S.K., Li, H., Meng, A., and Zhou, D. (2010). Total body irradiation causes residual bone marrow injury by induction of persistent oxidative stress in murine hematopoietic stem cells. *Free Radic. Biol. Med.* *48*, 348–356.
- Warren, L.A., and Rossi, D.J. (2009). Stem cells and aging in the hematopoietic system. *Mech. Ageing Dev.* *130*, 46–53.
- Warrick, E., Garcia, M., Chagnoleau, C., Chevallier, O., Bergoglio, V., Sartori, D., Mavilio, F., Angulo, J.F., Avril, M.-F., Sarasin, A., et al. (2012). Preclinical corrective gene transfer in xeroderma pigmentosum human skin stem cells. *Mol. Ther. J. Am. Soc. Gene Ther.* *20*, 798–807.
- Weksberg, D.C., Chambers, S.M., Boles, N.C., and Goodell, M.A. (2008). CD150- side population cells represent a functionally distinct population of long-term hematopoietic stem cells. *Blood* *111*, 2444–2451.
- Wheaton, W.W., and Chandel, N.S. (2011). Hypoxia. 2. Hypoxia regulates cellular metabolism. *Am. J. Physiol. - Cell Physiol.* *300*, C385–C393.
- Wijnhoven, S.W., Kool, H.J., Mullenders, L.H., van Zeeland, A.A., Friedberg, E.C., van der Horst, G.T., van Steeg, H., and Vrieling, H. (2000). Age-dependent spontaneous mutagenesis in Xpc mice defective in nucleotide excision repair. *Oncogene* *19*, 5034–5037.
- Wilson, A., and Trumpp, A. (2006). Bone-marrow haematopoietic-stem-cell niches. *Nat. Rev. Immunol.* *6*, 93–106.
- Wilson, A., Murphy, M.J., Oskarsson, T., Kaloulis, K., Bettess, M.D., Oser, G.M., Pasche, A.-C., Knabenhans, C., Macdonald, H.R., and Trumpp, A. (2004). c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. *Genes Dev.* *18*, 2747–2763.
- Wilson, A., Laurenti, E., Oser, G., van der Wath, R.C., Blanco-Bose, W., Jaworski, M., Offner, S., Dunant, C.F., Eshkind, L., Bockamp, E., et al. (2008). Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell* *135*, 1118–1129.
- Wood, R.D. (1997). Nucleotide excision repair in mammalian cells. *J. Biol. Chem.* *272*, 23465–23468.
- Wood, R.D., Mitchell, M., Sgouros, J., and Lindahl, T. (2001). Human DNA repair genes. *Science* *291*, 1284–1289.

- Wu, C.-H., van Riggelen, J., Yetil, A., Fan, A.C., Bachiredy, P., and Felsher, D.W. (2007). Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation. *Proc. Natl. Acad. Sci. U. S. A.* *104*, 13028–13033.
- Wu, X., Shell, S.M., Yang, Z., and Zou, Y. (2006). Phosphorylation of Nucleotide Excision Repair Factor Xeroderma Pigmentosum Group A by Ataxia Telangiectasia Mutated and Rad3-Related-Dependent Checkpoint Pathway Promotes Cell Survival in Response to UV Irradiation. *Cancer Res.* *66*, 2997–3005.
- Wu, Y.-H., Wu, T.-C., Liao, J.-W., Yeh, K.-T., Chen, C.-Y., and Lee, H. (2010). p53 dysfunction by xeroderma pigmentosum group C defects enhance lung adenocarcinoma metastasis via increased MMP1 expression. *Cancer Res.* *70*, 10422–10432.
- Xu, K., Wu, X., Tompkins, J.D., and Her, C. (2012). Assessment of anti-recombination and double-strand break-induced gene conversion in human cells by a chromosomal reporter. *J. Biol. Chem.* *287*, 29543–29553.
- Xu, X.S., Wang, L., Abrams, J., and Wang, G. (2011). Histone deacetylases (HDACs) in XPC gene silencing and bladder cancer. *J. Hematol. Oncol.* *4*, 17.
- Yamaguchi, M., and Kashiwakura, I. (2013). Role of Reactive Oxygen Species in the Radiation Response of Human Hematopoietic Stem/Progenitor Cells. *PLoS ONE* *8*, e70503.
- Yamamoto, R., Morita, Y., Oeohara, J., Hamanaka, S., Onodera, M., Rudolph, K.L., Ema, H., and Nakauchi, H. (2013). Clonal Analysis Unveils Self-Renewing Lineage-Restricted Progenitors Generated Directly from Hematopoietic Stem Cells. *Cell* *154*, 1112–1126.
- Yamazaki, S., Ema, H., Karlsson, G., Yamaguchi, T., Miyoshi, H., Shioda, S., Taketo, M.M., Karlsson, S., Iwama, A., and Nakauchi, H. (2011). Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. *Cell* *147*, 1146–1158.
- Yang, J., Xu, Z., Li, J., Zhang, R., Zhang, G., Ji, H., Song, B., and Chen, Z. (2010). XPC epigenetic silence coupled with p53 alteration has a significant impact on bladder cancer outcome. *J. Urol.* *184*, 336–343.
- Ying, Q.L., Nichols, J., Chambers, I., and Smith, A. (2003). BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell* *115*, 281–292.
- Van Zant, G., and Liang, Y. (2003). The role of stem cells in aging. *Exp. Hematol.* *31*, 659–672.
- Zhang, H., Gao, J., Ye, J., Gong, Z., and Gu, X. (2011a). Maternal origin of a de novo microdeletion spanning the ERCC6 gene in a classic form of the Cockayne syndrome. *Eur. J. Med. Genet.* *54*, e389–e393.
- Zhang, J., Niu, C., Ye, L., Huang, H., He, X., Tong, W.-G., Ross, J., Haug, J., Johnson, T., Feng, J.Q., et al. (2003). Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* *425*, 836–841.
- Zhang, S., Yajima, H., Huynh, H., Zheng, J., Callen, E., Chen, H.-T., Wong, N., Bunting, S., Lin, Y.-F., Li, M., et al. (2011b). Congenital bone marrow failure in DNA-PKcs mutant mice associated with deficiencies in DNA repair. *J. Cell Biol.* *193*, 295–305.

Zhang, X., Sejas, D.P., Qiu, Y., Williams, D.A., and Pang, Q. (2007). Inflammatory ROS promote and cooperate with the Fanconi anemia mutation for hematopoietic senescence. *J. Cell Sci.* *120*, 1572–1583.

Zhang, Y., Wang, X., Zhang, W., and Gong, S. (2013). An association between XPC Lys939Gln polymorphism and the risk of bladder cancer: a meta-analysis. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* *34*, 973–982.

Zharkov, D.O. (2008). Base excision DNA repair. *Cell. Mol. Life Sci.* *65*, 1544–1565.

Zhou, S., Schuetz, J.D., Bunting, K.D., Colapietro, A.M., Sampath, J., Morris, J.J., Lagutina, I., Grosveld, G.C., Osawa, M., Nakauchi, H., et al. (2001). The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat. Med.* *7*, 1028–1034.

ANNEX 1

Research article in preparation

XPC Affects Hematopoietic Stem Cell Maintenance *In Vivo*

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Short title: **XPC in HSC**

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Abstract

During hematopoiesis, the integrity of hematopoietic stem cells (HSC) is threatened by various stressors provoking accrual of DNA damage and in consequence perturbation in their functionality and maintenance. Nucleotide excision repair (NER) has been previously reported to be involved in HSC long-term repopulation ability. Xeroderma pigmentosum group C (XPC) protein, known to serve as a key DNA damage recognition factor in global genome repair (GGR), a sub-pathway of NER mechanism, has been recently reported to have preponderant roles outside of DNA repair in metabolism, oxidative stress and cell cycle. Here, we investigate the importance of GGR in HSC maintenance by the study of XPC function in human CD34⁺ umbilical cord blood (UCB) cells. Our data show that XPC protein is expressed in HSC and its expression is higher in CD34⁺ cells compared to mature CD34⁻ cells. In addition to the traditional 125 kDa protein, we noticed two XPC bands of lower molecular weights, 100 and 110 kDa. Additionally, XPC expression decreased concomitantly with decrease in amount of CD34⁺ cells in liquid culture for 14 days. Furthermore, XPC knockdown (KD) did neither affect the stability of CD34⁺ cells during liquid culture nor their progenitor ability. However, XPC KD impeded the long-term repopulation ability of CD34⁺ cells during NSG mice xenotransplantation in primary and secondary recipients leading to impairment of human cell engraftment and lineage reconstitution. Markedly, XPC KD has depleted the stem cell compartment *in vivo*. Overall, our data demonstrate a key role for XPC in maintenance of human HSC while being dispensable for mature cell.

Key words: XPC, HSC, CD34, maintenance, KD.

Introduction

HSC reside at the apex of hematopoietic hierarchy initiating hematopoiesis through a progressive production of more committed progenitors and eventually mature blood cells (Bryder et al., 2006). HSC are characterized by their unique self-renewal potency, by their ability to differentiate into all blood lineages, and by their quiescence (Kondo et al., 2003). The intricate balance between these stem cell properties is required to maintain hematopoietic homeostasis throughout adult life (Boisset and Robin, 2012), to insure immune function, and to respond to any sort of tissue injury and frequent DNA damage caused by various environmental and metabolic insults (Schlessinger and Van Zant, 2001; Van Zant and Liang, 2003). However, HSC characteristics represent a double-edged sword because its quiescent state renders them intrinsically vulnerable to accumulation of DNA damage (Niedernhofer, 2008) and their self renewal and differentiation state permits them to develop mutagenesis and eventually leukemia (Barnes and Melo, 2006; Reya et al., 2001).

To avoid impairment of function and maintenance, HSC are reported to be naturally equipped with multiple DNA damage response pathways that trigger DNA repair, cell cycle arrest, and apoptosis (Hollander et al., 2005). Based on the type of DNA damage occurring in mammalian cells, DNA repair pathways can be categorized into NER, base excision repair, mismatch repair, homologous recombination repair and non-homologous end joining (Bernstein et al., 2002). Among the several DNA repair pathways, NER pathway is reported to be particularly modulated in hematopoietic cells with differentiation (Hsu et al., 2007). NER is also demonstrated to be implicated in HSC maintenance where they showed that HSC functional capacity but not number was severely affected under conditions of stress in *Xpd* knockout (KO) mice, leading to the loss of reconstitution and proliferative potential, diminished self-renewal, increased apoptosis and, ultimately, functional exhaustion and this effect was enhanced with aging (Niedernhofer, 2008; Rossi et al., 2007). Furthermore, diminished ability of HSC to proliferate and self-renew in NER-deficient mice that were not exposed to exogenous genotoxic stress revealed that age-dependent spontaneous or endogenous DNA damage accumulation is responsible for the loss of HSC function (Niedernhofer, 2008; Rossi et al., 2007).

NER is defined as a versatile pathway for repairing helix-distorting lesions such as intrastrand cross-links, cyclobutane pyrimidine dimers (CPD), pyrimidine pyrimidone photoproducts (6-4PP) and bulky adducts produced mostly by ultraviolet (UV) and ionizing radiations (Rezvani et al., 2007), alkylating agents and chemicals. NER proceeds through two distinct

yet overlapping pathways: transcription-coupled repair (TCR), which specifically removes lesions from the transcribed strand of active genes, and global genome repair (GGR), which removes lesions throughout the genome (Hanawalt, 2002). Malfunctioning of the NER pathway may provoke several human genetic disorders including Xeroderma Pigmentosum (XP), for which seven NER-deficient genetic complementation groups (XP-A to -G) have been identified. It is known that XP patients exhibit extreme sensitivity to UV, high risk (1000 times) of developing skin cancer in childhood, and interestingly an increased risk (10 to 20 times) of developing internal cancers which is unrelated to UV exposure including several cases of leukemia (Kraemer et al., 1987). Markedly, unlike most XP complementation groups, XPC patients, defective in *XPC* gene, show a defect only in GGR but TCR is normal (Boon et al., 2004). The XPC protein functions as DNA damage recognition factor for GGR in which it recognizes structural DNA abnormalities associated with damaged bases. This provokes the binding of DNA helicase complex that unwinds the DNA helix. Thereafter the region with the lesion is incised by 3' and 5' endonucleases permitting its release. Finally, the gapped DNA lesion is restored by DNA polymerase and DNA ligase (Cleaver, 2005; de Laat et al., 1999; Rezvani et al., 2010).

Outside of XPC popular function as a DNA damage sensor in NER pathway, several studies held both on human and mouse models have revealed new functions of XPC mainly in cell metabolism and oxidative stress. For instance, *Xpc KO* mice showed divergent tumor spectrum compared to other NER deficient mouse models (Melis et al., 2008). XPC protein has been shown to prevent the occurrence of bladder cancer (Chen et al., 2007) and its polymorphisms were associated with increased lung cancer risk (Hu et al., 2005; Lee et al., 2005; Marín et al., 2004). Further studies by Rezvani and his colleagues showed that *XPC KD* in human keratinocytes leads to activation of NADPH oxidase-1 (NOX-1) that elevates the level of reactive oxygen species (ROS) thereby causing oxidative stress which will lead to spontaneous accumulation of mitochondrial and nuclear damage. In addition, subcutaneous injection of *XPC KD* cells into immunodeficient mice lead to development of squamous cell carcinoma (Rezvani et al., 2011a, 2011b). Accordingly, measurement of ROS indicated higher levels in *XPC KD* cells relative to control fibroblasts (Fréchet et al., 2008a). In addition, XPC function has been shown to be implicated in cell cycle control including checkpoint regulation and apoptosis (Stout et al., 2005; Wang et al., 2004). Latest reviews on XPC by Melis and his colleagues highlighted that XPC has several roles outside of NER pathway in cell cycle control, redox homeostasis, metabolism, and in BER pathway where it responds to oxidative DNA damage (Melis et al., 2011, 2013). These preponderant implications of XPC in damage

recognition, ROS generation, cancer, metabolism and ROS generation suggests that XPC could also have a potential role in HSC maintenance.

Interestingly, several studies have handled possible implication of XPC in the hematopoietic system. For instance, one study showed myelosuppression in an XPC patient (Salob et al., 1992). Fisher et al. have studied BM of an *Xpc KO* mice model subjected to carboplatin which, like cisplatin, produce a DNA damage spectrum, mainly 1,2 and 1,3 platinum diadducts that are substrates for NER *in vitro* (Shivji et al., 2006; Trego and Turchi, 2006). They found that *XPC*-deficient BM cells in comparison to wild type had an increased sensitivity to carboplatin by 10-folds, had a defective G1 checkpoints but an increased accumulation in G2, decreased colony-forming even in the absence of carboplatin (Fischer et al., 2009). Furthermore, pharmacogenomics studies showed implication of XPC in leukemia. For instance, Strom et al. correlated *XPC* Ala499Val polymorphisms with AML where he showed that patients carrying one or two variant alleles were associated with 78% increased mortality compared to the wild-type genotype (CC) (HR 1.78, $p = 0.02$) (Strom et al., 2010). Moreover, Guillem et al. correlated both *XPC* Ala499Val and *XPC* Lys939Gln polymorphisms with response of CML patients to imatinib treatment where he showed that the wild-type haplotype (499C-939A) was associated with a better response to imatinib in comparison to heterozygous carriers of TA/CC haplotype (Guillem et al., 2010).

In this current study, we characterize expression of XPC in human UCB CD34⁺ cells and its modulation with differentiation. We also report the effect of *XPC* silencing on UCB CD34⁺ cells maintenance *in vitro* and *in vivo*. Therefore, we have shown that XPC protein is highly expressed in CD34⁺ cells compared to the more mature progenitors. The cell culture, RNA interference strategy and methylcellulose assays demonstrated that XPC didn't impact the mature fraction of the hematopoietic system. However, using NSG model and reciprocal transplantation strategy, we present evidence that *XPC* KD leads to progressive diminution in stem cell pool.

Material and Methods

Purification of CD34⁺ cells from UCB

UCB samples were obtained from normal full-term deliveries with signed informed consent. Blood samples were collected in Dulbecco's Phosphate Buffered Saline (DPBS, Eurobio) medium supplemented with EDTA, Heparin choay (Sanofi Aventis, Paris, France), and Penicillin/Streptomycin (Gibco Life TechnologiesTM, USA) and were processed within 24 to 48 h. MNCs were collected by FicollTM density gradient media (Lymphocyte separation medium LSM 1077, Eurobio, Les Ulis, France). CD34 cell enrichment was then achieved by EasySepTM positive selection (Stem Cell Technologies, Vancouver, Canada). Finally, isolated CD34⁺ cells were counted and labeled with mouse anti-human CD34 antibody conjugated to phycoerythrin (cat# 12-0349-42, CD34-PE, eBioscience) to check for the purity. Isotype control was mouse immunoglobulin-G (IgG1 k) conjugated to PE (cat# 12-4714-82, Iso-PE, eBioscience). Flow analysis was performed on FACS CantoIITM (Becton Dickinson, San Jose, CA, USA) using Diva software. Only CD34⁺ cells with a purity of more than 90% were used in our experiments.

Production of lentiviral particles

The plasmids previously prepared in the laboratory are the following: pTPW-Ef1alpha-GFP-deltaU3-H1-shXPC-U5 for shXPC vector and pTPW-Ef1alpha-GFP-deltaU3-H1-shLuc-U5 for shLuc vector. Plasmids were amplified by maxiprep (Nucleo Bond® Xtra Maxi EF, Macherey-Nagel) according to the manufacturer instructions, quantified by Nanodrop®, and their quality was verified by restriction enzyme digestion. Lentiviral particles were produced by transient transfection of HEK (human embryonic kidney) 293T cells, using a calcium phosphate transfection technique. HEK 293T adhesion cell lines were cultured in DMEM medium with 10% fetal bovine serum (FBS) and penicillin/streptomycin (P/S; 10 U/mL). One million 293T cells were plated on 0.01% L-polylysine (cat# P-4832, Sigma) coated 10-cm plates and incubated at 37 °C for 3 days. When cell confluency reached 50-70% at day 3, the medium was changed (DMEM, 10% FBS, PS). Prior to transfection, 2.5 M Calcium chloride solution and 2x-HBS HEPES buffered saline buffer of exact pH = 7.12 are prepared. The latter consists of 280 mM sodium chloride, 1.5 mM sodium phosphate, and 100 mM HEPES. 24 h later, 293T cells were washed then transfected by calcium phosphate technique, using multiple constructs for each petridish: the packaging construct pCMVPAX2 (10 µg), a vesicular stomatitis virus glycoprotein (VSVG) envelope construct pMD.G (4 µg) and a

vector construct (shXPC or shLuc, 10 μg). After 8 h of transfection, the medium was removed and replaced by serum-free Optimem (Gibco, CergyPontoise, France) supplemented with 2 mM HEPES (Fluka, Lyon, France) and 10 U/mL P/S (Lonza, Basel, Switzerland). 48 h later, viral supernatants were collected into 50 mL falcon tubes, centrifuged to remove the cell debris, filtered through 0.22 μm filters, and concentrated by ultracentrifugation at 35000 $\times g$ for 4 h at 4 °C. They are then collected in 200 to 400 μL of StemSpan medium, suitable for HSC culture. Infectious titers were determined by transducing 293T cells with serial dilutions of viral supernatant going from 1/1000 (using 1, 3 and 10 μL per well) to 1/100 (using 3 and 10 μL per well). For transduction, 5×10^4 293T cells, per well of a 24 well plate, were incubated for 15-16 h with the appropriate volume of lentiviruses in a total volume of 200 μL of DMEM supplemented with 10% FBS-PS. The supernatant was then removed and replaced by 500 μL of the same medium. After three days, the titer was determined by checking the percentage of GFP⁺ cells by flow cytometry. Virus titer (PI/ml) was calculated as equal to %transduction \times initial number of cells \times dilution factor \times 1000.

Culture of CD34⁺ cells

CD34⁺ cells were thawed in FBS (cat# CVFSVF00-01, Eurobio) medium, and cultured in STEM SPANTM serum-free medium (cat# 09650, SFEM, StemCell Technologies, Canada), supplemented with 50 ng/mL Stem Cell Factor (cat# 300-07, SCF, Peprotech, USA), 50 ng/mL Fms-related tyrosine kinase 3 ligand (cat# 300-19, Flt3-L, Peprotech, USA), 50 ng/mL Thrombopoietin (cat# 300-18, TPO, Peprotech, USA), 1% P/S (10 U/ml) and 1 μM of aryl hydrocarbon receptor antagonist Stem Regenin 1 (cat#1967-1, SR1, Biovision Incorporated, Milpitas, CA95035, USA). Cells were cultured at 37 °C in humidified atmosphere with 20% O₂ and 5% CO₂ for 14 days. At different time points (D4- D7- D11- D14), cells were collected, pelleted and stored at -80° C for further molecular analysis.

Transduction of CD34⁺ cells

CD34⁺ cells were transduced with shXPC and shLuc vectors using a M.O.I of 100. The cells were cultured in complete BIT medium (cat#09500, Bovine serum albumin/Insulin/Transferrin, BIT 9500 Serum Substitute, Stem CellTM Technologies) supplemented with necessary cytokines at concentration of 10⁶ cells/mL for 15-16 h at 37 °C in a fully humidified atmosphere (20% O₂ and 5% CO₂). Later, cells were washed twice with

IMDM and resuspended in complete Stem Span medium at concentration 10^5 cell/mL. After four days of transduction, the percentage of GFP-expressing cells was determined by flow cytometry.

GFP stability assay

CD34⁺ cells transduced 50% with shXPC and shLuc vectors were put in culture for two weeks. The amplification of cells was assessed by trypan blue exclusion method and the GFP stability was assessed by flow cytometry that allowed detection of GFP-expressing cells.

Progenitor cell assay

Committed progenitors were assayed by seeding 500 transduced CD34⁺ cells in semi-solid culture medium with recombinant cytokines known as StemAlpha 1D (cat# 5112, Stem Alpha SA, La Chenevatiere, France). Cells were seeded after one day of culture of transduced CD34⁺ cells with shXPC or shLuc lentiviruses. Colonies were scored according to their morphology at 14 days following plating using an inverted microscope.

Cell sorting

FACS Aria (Becton Dickinson) machine was used to sort GFP-expressing cells after four days of CD34⁺ transduction with shLuc and shXPC vectors. After sorting (D4), CD34⁺ cells were cultured for an additional ten days and the percentage of CD34⁺ cells (and GFP⁺) determined each three days (D7, D11, D14). Also we attempted to sort CD34-expressing cells and CD34-deficient cells (CD34⁺/CD34⁻) after 11 days of liquid culture of CD34⁺ cells then pellet the cells for WB analysis and qRTPCR.

Western blot

Cells were lysed using RIPA buffer which constituted of 50 mM Tris-cl pH7.5, 150 mM NaCl, 1% triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and 0.1 mM PMSF along with cocktail of protease inhibitors. The extracted proteins are quantified using 'Pierce[®] BCA Protein Assay' kit (ThermoScientific, Rockford, USA) according to the manufacturer's recommendations. Protein samples were prepared in a loading Laemmli

Sample buffer which constituted of 60 mM Tris-Cl pH 6.8, 2% SDS, 10% glycerol, 5% β -mercaptoethanol and 0.01% bromophenol blue buffer before separation by SDS-PAGE electrophoresis. Total protein extracts (25 μ g) were loaded into the wells of stacking gel which constitutes of 30% acrylamide solution, 1 M Tris pH 6.8, 10% SDS, 10% ammonium persulfate and TEMED. Then proteins were migrated on 8% SDS polyacrylamide gel which constitutes of 30% acrylamide, 1.5 M Tris pH 8.8, 10% SDS, 10% ammonium persulfate and TEMED. Gels were then transferred by wet transfer to nitrocellulose membranes at room temperature at constant 100 volts during 1h30min. The membrane was then blocked in 5% fat-free milk prepared in 0.1% Tween20-TBS for 1 h at RT. Detection of the protein of interest is done by probing the membrane with monoclonal antibodies (MAb) overnight at 4°C. Mouse MAb anti-XPC (clone 3.26, cat# GTX 70294, GeneTex) directed against C-terminal of XPC was used at a 1:1000 dilution. Rabbit polyclonal anti-XPC (cat# GTX 70308, GeneTex) antibody directed against N-terminal of XPC was also used at a 1:1000 dilution. Actin was used as control for equal loading by the use of Mouse anti-Actin MAb used at a 1:10,000 dilution. XPC and Actin were revealed after secondary incubation for 2 h with mouse or rabbit anti-immunoglobulin horseradish peroxidase (HRP)-linked antibody used at dilution of 1:5000 and 1:10,000 respectively. Blots were developed using the chemiluminescence ECL reagent Kit (Bio-Rad). Quantification of XPC and actin bands was carried out by densitometry (FujifilmTM LAS-3000 Imager).

Quantitative Real-Time PCR (qRT-PCR)

Total cellular RNA was extracted using Trizol (Invitrogen, CA) according to the manufacturer's instructions. The quantity of RNA is assessed for by Nanodrop. RNA is said to be pure if value of $OD_{260/280} \geq 1.8$. Total cellular RNA (500 ng) was reverse transcribed using the Transcription First Strand cDNA synthesis kit (Roche Version 6, Germany). qRT-PCR was carried out for XPC and tubulin using Sybr Green QPCR Master Mix (Agilent Technologies, USA) with an ABI Thermal cycler. The reactions were cycled 40 times after initial polymerase activation (95 °C, 3 min) using the following parameters: denaturation at 95 °C for 15 s, annealing at 60 °C for 22 s. A final fusion cycle (95 °C, 1 min; 60 °C, 30 s; 95 °C, 30 s) terminated these reactions. The sequence of primers used to detect expression of *XPC* is F: GGTGAGGCTTGGAGAAGTAC for Forward primer and R: ACATTCCCAAACCTCGTTCCG for Reverse primer. The sequence of primers used to detect expression of house-keeping gene *tubulin* is F: GAGTGCATCTCCATCCACGTT for Forward primer and R: TAGAGCTCCCAGCAGGCATT for Reverse primer.

NOD-scid IL2Rg^{null} mice repopulation assay

NOD-scid IL2Rg^{null} (NSG) mice were purchased from Jackson Laboratory (Bar Harbor, Maine, USA) and bred in specialized animal house in University of Bordeaux. The NSG mice used in the experiment were 7- to 10-week old. Prior to transplantation, mice received a preparative conditioning regimen consisting of a 3-day intra-peritoneal injection of 22 mg/kg Busilvex® (Pierre Fabre, Boulogne, France). Mice were retro-orbital injected with 80000 transduced CD34+ cells (shXPC or shLuc) at 10⁵cell/100ul. Bone marrow samples from the right femur (RF) were later aspirated, under isoflurane anesthesia, at 3, 6 and 12 weeks after transplantation. To avoid possible pain, mice received Vetegesic® (Cetravet SA Cooperative, Dinan, France) at 100 µg/kg. Human cell engraftment and hematopoietic composition were assessed by flow cytometry. At 18-24 weeks, primary mice are sacrificed, BM cells from left and right tibia, femur and pelvis are pooled in which a part is sacrificed for analysis of hematopoietic composition by flow cytometry whereas the rest of cells are intravenously injected into busilvex-preconditioned secondary recipients. Only mice with more than 5 percent human transduced GFP positive cells were used for secondary transplantation. Moreover, peripheral blood collected by heart puncture under anesthesia, spleen and thymus are analyzed from the primary and secondary recipients after sacrifice. All animal work was done under the approval of the ethical committee board of University of Bordeaux.

Labeling of BM cells

Human CD45-PC7 (cat# 25-0459-42, eBioscience), mouse CD45-eFluor450 (cat# 48-0451-82, eBioscience) and Ter119-eFluor450 (cat# 48-5921-82, eBioscience) antibodies allowed us to distinguish human cells from the mouse cells and hence to determine the percentage of engraftment. Erythroid lineage was studied by the use of CD36-PE (cat# 555455, BD Pharmingen) and GPA-APC (cat# 17-9987-42, eBioscience) antibodies. Myeloid lineage was studied by the use of CD33-APC (cat# 551378, BD Pharmingen), Mac1-PE (cat# 555388, BD Pharmingen), and CD14-APCVio770 (cat# 130-096-622, MACS) antibodies. Lymphoid lineage was studied by the use of CD3-APC (cat# 17-0038-42, eBioscience), CD19-PE (cat# 555413, BD Pharmingen), CD4-APCVio770 (cat# 130-096-652, MACS) and CD8-Viogreen (cat# 130-096-902, MACS) antibodies. Stem cells were studied by primary incubation with human lineage/biotin antibody cocktail (cat# 120-001-875, MACS) used to mark all lineage-positive mature cells allowing us to gate for the lineage-negative rare stem cells, followed by secondary incubation with Antibiotin-Vioblue (cat# 130-094-669, MACS), CD34-PE (cat#

12-0349-42, eBioscience), CD38-APC (cat# 555462, BD Pharmingen), and CD45RA-Viogreen (cat#130-096-921, MACS) antibodies. CD16/32 antibody was used in all our panels to block non-specific binding of antibodies. In addition, panel of isotype antibodies was used to help locate the gates and constituted of Rat IgG2bk Iso-eFluor450 (cat# 48-4031-82, eBioscience), Mouse IgG1K Iso-APC (cat# 17-4714-82, eBioscience), Iso-PC7 (cat# 25-4714-42, eBioscience) and Iso-PE (cat# 12-4714-82, eBioscience). 10^5 cells were used for FACS acquisition for each panel. Compensation was automated using BD FACSDiva software using negative control compensation particles.

ROS measurement on Lin⁻CD34⁺GFP⁺ BM cells from NSG mice

One million BM cells from each NSG mice were used to measure ROS production in human transduced stem cells Lin⁻CD34⁺GFP⁺ after 18 weeks of transplantation. First BM cells were incubated with 2.5 μ M CellROX probe (cat#C10422, Life Technologies) for 20 min at 37°C. Directly biotin-lineage antibody is added to the cells to label all lineage-positive cells while incubation for 20 min at 4°C. Cells were washed with PBS and were secondary labeled with antibiotin-vioblue and CD34-PE antibodies during incubation for 20 min at 4°C. Cells were re-washed and analyzed on FACS machine.

Statistical analysis

Data is presented either as the mean \pm S.E.M. or as the mean \pm standard deviation (SD). Statistical analysis was performed using the two-tailed student's t-test for comparison of two groups to determine the level of significance. Results were considered significant when p-value < 0.05.

Results

XPC expression relies on hematopoietic differentiation state

The XPC expression in mouse HSC and its downstream progenitor cells obtained by large-scale microarray analysis has been recently documented (Beerman et al., 2014). Interestingly XPC expression was higher in the progenitor cells (MPP, CLP and BLP) in comparison to the HSC demonstrating a regulation of expression during differentiation and certainly associated with DNA repair in the hematopoietic system. However, none of the previous studies reported the XPC expression in different human hematopoietic compartment. Therefore, XPC expression, at both the transcriptional and translational levels, has been investigated in stem/progenitor cell and compared to expression in the mature cell fraction based on the expression of the CD34 marker, CD34⁺ and CD34⁻ respectively. The analysis of XPC mRNA level showed a higher expression in CD34⁺ cells (3.1 ± 0.5 folds) in comparison to CD34⁻ cells (**Figure 1A**). After that, in order to study the protein expression of XPC in CD34⁺ and CD34⁻ fractions, we used as positive control skin-derived keratinocytes that are known to express XPC (Rezvani et al., 2010). We observed that all CD34⁺ cells expressed XPC whereas it is more inconsistently expressed in CD34⁻ cells (**Figure 1B**). Surprisingly, compared to keratinocytes, WB analysis on hematopoietic cells revealed a different pattern of expression. While the expected 125 kDa band is observed as in keratinocytes, additional bands with lower apparent molecular weight were observed in hematopoietic cells (about 100 and 110 kDa). The protein quantification, taking into consideration all the bands, shows significant higher expression of XPC protein (13.8 ± 8.4 folds) in CD34⁺ cells in comparison to CD34⁻ cells (**Figure 1C**). Further analysis of the three XPC bands shows that the 110 and 125 kDa bands expression is mostly linked to the immature state of the cells, CD34⁺ cells having 2.8 ± 1.0 and 5.1 ± 1.1 folds more expression than CD34⁻ respectively. In contrary, the 100 kDa band is less abundant in CD34⁺ cells than CD34⁻ cells by 0.48 ± 0.08 folds (**Figure 1D**). Hence, XPC is upregulated in CD34⁺ cells in comparison to CD34⁻ cells at both the transcriptional and the translational level.

Being interested by the bands, we intended to determine whether the bands are not related to individual variation such as polymorphisms and alternative splicing. To do that, we performed protein analysis on keratinocyte from different individual samples. A unique XPC band has been observed in all keratinocyte samples, corresponding to 125 kDa band although it is possible to see extra but faint bands in samples S2, S4 and S8 (**Supp 1A**). This confirms that

the appearance of the three bands of XPC is particular to the hematopoietic cells. However, to exclude unspecific recognition by antibody, which targets the C-terminal region, an anti-XPC antibody that recognizes the N-terminal region has been used. Both antibodies detect the same XPC bands (**Supp 1B**).

XPC expression decreases throughout CD34⁺ culture

In order to better relate the XPC expression and the differentiation status, we performed *in vitro* culture of CD34⁺ cells and followed the molecular weight. First of all, we have found that the global XPC expression decreases with time (**Figure 2A-B**) by up to 88% after two weeks of culture. In parallel, the percentage of CD34⁺ cells was followed and used as a differentiation indicator (**data not shown**). Data clearly show that the loss of CD34⁺ cells is accompanied by downregulation of XPC. Interestingly, the expression of the different XPC protein bands was also modified according to the loss of CD34 expression. Results show decrease of the 125 kDa band (0.34 ± 0.10 folds) concomitant to increase of the 100 kDa band (1.9 ± 0.3 folds) after 14 days of culture (**Figure 2C**).

To further confirm that the residual expression of XPC is restricted to CD34⁺ cells in culture, CD34⁺ cells were cultured until day 11 and sorted based on the CD34 expression. Data revealed a quasi-exclusive XPC expression in CD34⁺ cells (**Figure 2D**). Quantification of the total bands showed a much higher expression of XPC (42 ± 30 folds) in purified CD34⁺ compared to CD34⁻ fraction (**Figure 2E**).

XPC is dispensable for maintenance of CD34⁺-derived cells in culture

In order to study whether XPC might affect hematopoietic cell behavior, we attempted to knockdown its expression in CD34⁺ cells using lentiviral vectors that carry interfering RNA targeting *XPC* (**Supp 2A**). To determine the inhibition efficacy at a low copy number of the lentiviral vector, cells were transduced at 20 to 30 % and sorted based on GFP expression. WB analysis shows that all XPC bands were extinguished (71 ± 3.5 % reduction) demonstrating the efficiency of the vector in CD34 cells (**Figure 3A-B**). The effect of *XPC* KD on the maintenance of CD34⁺ cells has been first investigated in liquid culture. The amount of GFP⁺ cells remained comparable in both conditions (**Figure 3C**). In addition, data show that both the proliferative and differentiation potential of CD34⁺ cells has not been affected by *XPC* KD (**Supp 2B-C**).

Furthermore, the function of CD34⁺ cells can be assessed by their ability to produce hematopoietic progenitors. In order to investigate whether *XPC* KD can affect the amount of

progenitors produced by the transduced CD34⁺ cells, shXPC- and shLuc-transduced CD34⁺ cells were seeded in semi-solid methylcellulose medium. Data did not show any significant difference between XPC KD cells and control in terms of percentage of expressing cells suggesting that XPC is not required for progenitor repopulating ability (**Figure 3D**). In addition, no difference has been observed between myeloid or erythroid progenitors (**Figure 3E**). Thus, XPC KD does not seem to impact CD34⁺ differentiation.

XPC is crucial *in vivo* to sustain human hematopoiesis

In order to test the role of XPC on stem cell maintenance, it was essential to perform *in vivo* experiments. Therefore, xenotransplantation of CD34⁺ cells transduced with the shXPC vectors were performed into immune-deficient NOD/SCID mice. The human cells were followed over the time by the percentage of CD45⁺ cells in bone marrow aspirates and the transduced cells by the GFP-expression (**Figure 4A**). Our data showed decreased percentage of GFP⁺ cells with time in XPC KD cells compared to the control, thus indicating depletion of CD34⁺ cells *in vivo* and suggesting a putative role of XPC in the stem cell maintenance or proliferation of the committed progenitors (**Figure 4B**). Then, to demonstrate that the decrease was not due to a change in the distribution of cells in the different hematopoietic tissues, we checked the engraftment of the transduced cells in the BM, PB, spleen and thymus of these mice after 18 weeks of transplantation. Thus, we noticed a similar decrease in engraftment in each of the different tissues (**Figure 4C**).

In order to determine whether XPC might affect hematopoietic lineages differently we followed the expression of the GFP in the different subpopulation of huCD45⁺ cells. To analyze the erythroid lineage, we have investigated GFP percentage in GPA⁺ cells and CD36⁺ cells after 18 weeks in which we obtained a highly significant decrease in the ratio of GFP⁺ cells relative to day 4 in XPC KD mice relative to shCtrl mice. To analyze the myeloid lineage, we have investigated GFP percentage in CD33⁺ and CD11b⁺CD14⁺ cells in the BM of our mice 18 weeks post transplantation. We found that the ratio of CD33⁺GFP⁺ cells and CD11b⁺CD14⁺GFP⁺ cells relative to day 4 decreased significantly after 18 weeks of transplantation in XPC KD mice in comparison to shCtrl-transduced mice. To analyze the lymphoid lineage, we have investigated GFP percentage in CD19⁺ and CD3⁺ cells, representing B- and T-lymphocytes respectively; in the BM of our mice 18 weeks post transplantation. The ratio of CD19⁺GFP⁺ cells and CD3⁺GFP⁺ cells have revealed strong decrease in XPC KD mice compared to shCtrl-transduced mice (**Figure 4D**).

XPC silencing affects maintenance of stem cells

To explain the decrease in mature lineage we could hypothesize similar general mechanism in all the lineages or impact on the stem cell or early progenitors. To uncover this we determine the percentage of GFP cells within stem cell fractions according to the expression of various markers that are CD34, CD38, CD45RA. Thus, we attempted to check for the percentages of the stem cells subpopulations, ranging from the most immature stem cell population identified as Lin⁻CD34⁺CD38⁻CD45RA⁻ to the more mature Lin⁻ cells, present in the bone marrow of these mice 18 weeks post transplant. The detection of the %GFP⁺ cells in the BM cell populations following different degrees of maturity has revealed a strong decrease of GFP in *XPC* KD cells compared to shCtrl-transduced populations. Interestingly, all the stem cell subpopulations showed significant decrease in %GFP in *XPC* KD mice compared to shCtrl-transduced mice. Therefore *XPC* KD affects the stem cell maintenance in general and affects the Lin⁻CD34⁺CD38⁻ population of cells in particular (**Figure 5A**).

To further confirm the requirement of XPC for proper functioning of the stem cells, primary BM cells have been transplanted in secondary recipients and GFP expression determined in human cells. Data show remarkable decrease (76.7% ± 11.3) in the percentage of engrafted *XPC* KD CD34⁺ cells whereas not or few decrease has been noticed for control cells (**Figure 5B**). The further reduction in the percentage of *XPC* KD cells observed at 18 weeks postsecondary transplantation as compared to the level obtained at 18 weeks post primary transplantation strongly demonstrates that the maintenance of human long-term repopulating cells with self-renewing ability is dependent on XPC.

To further explain the decline seen following the knockdown of *XPC*, we hypothesize an increase in the oxidative stress. Previous studies have showed that XPC is implicated in redox homeostasis and prevents oxidative DNA damage (Melis et al., 2011, 2013; Rezvani et al., 2011c). Indeed, the loss of XPC in keratinocytes triggers increased ROS production. Thus BM cells from the mice were harvested 18 weeks post-transplantation and hematopoietic stem/progenitor cells labeled using Lineage-depletion cocktail, anti-CD34 and the CellROX probe which detected a wide range of ROS. Our data did not reveal any significant difference in the oxidative stress in Lin⁻CD34⁺ cells obtained from both shLuc- and shXPC-transduced mice (**Figure 5C**). Therefore the depletion of *XPC* KD CD34⁺ cells in NSG mice is certainly not due to oxidative stress.

Discussion

Results showed that XPC is highly expressed in CD34⁺ in comparison to its CD34⁻ counterpart, which suggests that XPC is probably more required in stem cells than in its downstream mature cells. Consistent with this observation, XPC expression decreased when CD34⁺ cells have been cultured paralleling the decrease of the CD34⁺ cell percentage. Therefore the decrease in XPC expression was correlated with differentiation of HSC. In agreement with our data, other studies have reported that the differentiation of cells is accompanied with downregulation of NER in general. For instance, GGR was found to be downregulated in terminally differentiated neurons (Nospikel and Hanawalt, 2000, 2002). Moreover, differentiation of AML cell lines was accompanied by a strong attenuation in the repair of cisplatin intrastrand GTG crosslinks (Hsu et al., 2007). In addition, NER was recently found to be downregulated in myeloid cells during differentiation. Microarray analysis of 100 AML patients showed that XAB2 expression decreased during granulocyte differentiation and that the more differentiated cells were more susceptible to cisplatin than more immature myeloid leukemia cell lines (Aoki et al., 2014). Therefore, it is likely that GGR might be downregulated during differentiation of HSC into more mature cells which can explain our observed decrease in XPC expression. However, we can suggest also that XPC is more important in stem cells than in its downstream progenitors. However, Hyka-Nospikel et al. reported that NER is downregulated at the global genome level in quiescent human B-lymphocytes while maintaining proficient repair of constitutively expressed genes, which suggests the accumulation of DNA damage once B-lymphocytes are driven into proliferation. They have also found a molecular link between attenuation of NER during quiescence and cell mutagenesis in which NER attenuation in quiescent cells is associated with incomplete phosphorylation of the ubiquitin activating enzyme Ube1, a factor required for proficient NER (Hyka-Nospikel et al., 2011).

Normally, the XPC molecular weight is described as the 125 kDa band as shown in keratinocytes (Rezvani et al., 2010). Surprisingly, the traditional full length (125 kDa) XPC protein was accompanied by the expression of two shorter proteins of ~100 and 110 kDa. The 125 kDa protein was upregulated in CD34⁺ cells, in comparison to CD34⁻ cells, oppositely to the 100 kDa protein band. In addition, during liquid culture of CD34⁺ cells, which induced cell commitment, we have observed that the disappearance of the 125 kDa band whereas the other 2 bands were upregulated. The different protein forms of XPC in hematopoietic cells opened new speculations on the origin of three bands. The discrepancy between different bands might be explained by certainly transcriptional or/and post-transcriptional

modifications or/and degradation. A link between the modifications and the functional activity or resistance to various cell stresses could be also hypothesized. For instance, Warrick et al. reported three protein bands of XPC (125, 110, 100 kDa) only when keratinocytes were forced to overexpress XPC (Warrick et al., 2012). These authors have reported that these short forms of XPC did not seem to act as dominant negative regulator of NER or to modify cell behavior. Warrick and his team hypothesized that the three bands maybe mRNA splicing isoforms. Indeed, two in-frame AUG codons exist in the *XPC* coding sequence downstream the bona fide initiation codon and, theoretically, translation initiation from these codons could generate proteins of 823 (~110 kDa) and 747 (~100 kDa) amino acids, respectively. Furthermore, Legerski et al. reported a cloned human XPC complementary DNA that lacks the 117 first amino acids, presumably corresponding to the 110 kDa band. Interestingly, expression of this protein in immortalized XPC cells restored normal UDS levels and UV survival properties (Legerski and Peterson, 1992). Nevertheless, until now the function of the 100 kDa band remains ambiguous in which it has not been investigated previously. One classical modification is phosphorylation. For instance, XPC has been reported to be phosphorylated by ATM and ATR pathways under stress condition (Matsuoka et al., 2007; Wu et al., 2006). WIP-1, a serine/threonine wild-type p53-induced phosphatase 1, has been reported to negatively regulate NER by dephosphorylating XPC and XPA leading to its inactivation (Nguyen et al., 2010). Other post-translational modifications were reported in literature for XPC. XPC is ubiquitinated during the NER process by DDB2, which cause an increase in the affinity of XPC for DNA (Sugasawa, 2006; Sugasawa et al., 2005; Wang et al., 2007). Another example is that XPC can be sumoylated upon UV irradiation by SUMO-1 which is believed to stabilize XPC (Wang et al., 2005). Nevertheless, in our experiments it is not possible that XPC might be ubiquitinated or sumoylated since addition of ubiquitin or SUMO moiety will result in much bigger band. Indeed in Wang et al. article, the XPC bands that he found were of much higher molecular weight 145-220 kDa and he mentioned that if the modified XPC has a higher molecular weight which is at least 20 kDa more than that of native XPC the modifications are speculated to be ubiquitinylation or sumoylation (Wang et al., 2005). Notably, the size of one ubiquitin moiety is 8.5 kDa and one SUMO moiety is 12 kDa and normally the protein is covalently bounded with several moieties upon a posttranslational modification. Another possibility is that XPC might be degraded where it was reported that XPC degradation results by direct interaction with the proteasome (Wang et al., 2007).

The function of XPC has been investigated by knocking down *XPC* in CD34⁺ cells with a lentiviral vector expressing a shRNA directed against the *XPC*. The effect of the silencing has

then been evaluated by *in vitro* culture, in progenitor cell assay and in xenotransplantation. We showed that *XPC* KD neither affect the proliferation nor the CD34⁺ rate of *in vitro* cultured cells. Moreover, CFU assay did not reveal any effect of *XPC* KD on the clonogenic capacity of CD34⁺ progenitors. Likewise, *XPC* KD did not affect the number of myeloid and erythroid colonies pointing an absence for *XPC* effect on differentiation capacity.

As an explanation to our result, recent study by Beerman et al. revealed that quiescent HSC have attenuated expression of DNA damage repair and response genes among which NER pathway is remarkably downregulated in quiescent HSC. However, as HSC enter into cell cycle, they don't undergo cell death or inability to produce colonies *in vitro* or failure to reconstitute blood cells *in vivo* which was explained by that DNA repair pathways are upregulated when HSC enter cell cycle leading to repair of strand breaks (Beerman et al., 2014). Thus we can suggest that liquid culture of CD34⁺ cells promoted their differentiation into more mature cells, thus changing from quiescent state to cycling state, which has lead to upregulation of DNA repair pathways that have compensated for *XPC* KD in the cells.

Therefore effect in stem cells was challenged by xenotransplantation into immunodeficient NSG mice which provides a powerful assay system to characterize the HSC compartment (Bonnet, 2002). And since the production of different cell types in xenografts is temporally restricted in which not all lineages may be readily assayed at any given time point, we decided to follow a careful kinetic assessment of the engraftment. In primary mice transplants, our data showed a progressive depletion in human engrafted cells silenced for *XPC* expression that reaches significance after 12 weeks and a more dramatic drop was observed after 18 weeks. Further analysis of *XPC* effect on the human hematopoiesis revealed that *XPC*-silencing has impaired the erythroid, myeloid, lymphoid reconstitution lineage reconstitution after 18 weeks of transplant. Moreover, we found equivalent diminution in the frequency of the immature stem cell subpopulations, ranging from the immature Lin⁻CD34⁺CD38⁻ cells to the more mature Lin⁻CD34⁺, and Lin⁻ cells, in *XPC* KD mice after 18 weeks of transplantation. The prominent effect of *XPC* on stem cell maintenance was further confirmed by secondary transplantation which witnessed a major depletion in the quantity of human engrafted cells in the *XPC* KD mice. Thus, our data show modulation of *XPC* in hematopoietic cells and demonstrate that *XPC* participates to stem cell maintenance whereas this factor is not required for committed cell proliferation as initially suggested by the low expression in CD34⁻ cells. Conclusively, we have found that *XPC* is important in maintenance of HSC. In agreement with our data, *XPC* was reported to be implicated in embryonic stem cell maintenance through the direct interaction of *XPC*-RAD23B-CETN2 complex with Oct4 and Sox2. This interaction has been shown to be a requisite for embryonic stem cell self-

renewal and efficient somatic cell reprogramming (Fong et al., 2011). We suggest that the importance of XPC in the maintenance of human HSC could be due to its recently reported role in transcription. Indeed, Le May et al. has demonstrated that, under absence of exogenous genotoxic stress, XPC protein is indispensable for the assembly of NER factors to the transcription site which permits the achievement of an optimal DNA demethylation and histone posttranslational modifications thereby allowing an efficient RNA synthesis (Le May et al., 2010). Moreover, it is potentially possible that decline in number of CD34+ cells silenced for *XPC in vivo* could be due to the mechanism of apoptosis. Indeed, several studies has showed that DDR response in fetal HSC is mediated by the pro-apoptotic p53 and the pro-survival genes of the Bcl2 family (Asai et al., 2011; Seita et al., 2010; Weissman, 2000).

Previous studies have pointed to a role for XPC in repair of oxidative DNA damage. XPC has been shown to interact with several glycosylases such as 3-methyladenine DNA glycosylase (Miao et al., 2000), thymine DNA glycosylase (TDG) (Shimizu et al., 2003), selective monofunctional uracil DNA glycosylase (SMUG1) (Shimizu et al., 2003), and 8-oxoguanine DNA glycosylase (OGG1) (D'Errico et al., 2006). Moreover, XPC cells showed higher ROS levels compared to control fibroblasts (Fréchet et al., 2008b). Rezvani et al. demonstrated that XPC defect generates oxidative stress in the cell as measured by elevation of ROS levels (Rezvani et al., 2011a, 2011b). A recent study by Menoni et al. has showed that XPC had a role in protection of cells against oxidative DNA damage where it was rapidly recruited to oxidative DNA lesions in living cells (Menoni et al., 2012). Therefore, we have chosen to assess the ROS levels in stem/progenitor cell from NSG bone marrow. However, our study did not demonstrate increase in ROS in *XPC*-silenced stem/progenitor cell compared to normal cells. In support to our date, HSC are known to cycle very infrequently and primarily reside in the relatively inactive metabolic phase G0 of the cell cycle (Bradford et al., 1997), which exposes them to lower levels of damage-inducing metabolic side products and reactive oxygen species than more metabolically active differentiated cell types (Bryder et al., 2006). On the other hand, stem cells have been reported to express high levels of numerous ABC/MDR transporter genes (Zhou et al., 2001) whose products are known to have physiological roles in cytoprotection. Piccoli et al. pointed out the fact that HSC has to be pre-treated with cyclosporine A, an inhibitor of multi-drug resistant pump (MDR1) that allows the substantial trapping of the probe inside the cell. Hence, not using cyclosporine might allow the escape of ROS detection (Piccoli et al., 2005, 2007). However, it can be also argued that CellROX probe measure the total ROS level, which could be constant due to balance effect in the oxidative metabolism. Instead, we should have measured separately the cytosolic ROS and the mitochondrial ROS using more specific probes.

Conclusion

Overall, our data have evidenced the existence of different levels of expression as well as different expression profiles of the XPC protein in hematopoietic cells. Investigating the XPC expression profile, we have showed that the 125 kDa band, corresponding to the highest molecular weights, are mainly expressed in immature cells. Data highly suggest post-transcriptional modifications of the protein, which could be then associated with either stability or/and activity of the protein. Consistently, we showed decrease of XPC protein expression during CD34⁺ differentiation in culture. We have showed *in vitro* that XPC KD neither affects the proliferation and differentiation of the CD34-derived cells in liquid culture nor the ability of progenitor cells. However, we showed *in vivo* that XPC is involved in long-term hematopoietic capacity. All together, these data suggest a role of XPC in stem cell compared to committed/mature cells. However, it is likely wise to think that *in vitro* culture conditions might protect the cells from oxidative stress, and consequently might prevent their loss of function. However, *in vivo* loss of stem cells following XPC KD does not seem to be related to increase ROS levels. Further investigation should be held at the molecular level in order to find the pathways leading to the loss of long-term hematopoietic ability.

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

Figure Legends**Figure 1: XPC expression in human CD34⁺ and CD34⁻ fractions**

- A)** RNA from UCB-derived CD34⁺ and CD34⁻ samples were subjected to Q-RTPCR for assessment of XPC RNA expression in CD34⁺ and its CD34⁻ counterpart (n = 3). Values represent the average fold change of XPC expression in CD34⁺ cells, normalized to tubulin, and relative to CD34⁻ cells.
- B)** Total protein extracts from human CD34⁺ and its CD34⁻ counterpart were assessed for XPC protein expression by western blot. Actin was used as loading control. The arrowheads indicate the three XPC bands estimated at 100, 110 and 125 kDa. Keratinocytes from skin were used as positive control. S1, S2, S3, S4 represent four individual UCB samples.
- C)** Protein quantification analysis of the WB shown in A (n = 12). Values represent the average fold change of XPC expression in CD34⁺ cells, normalized to actin, and relative to CD34⁻ cells.
- D)** Protein quantification analysis of each XPC band (n = 12). Values represent the ratio of each XPC band to total XPC bands in CD34⁺ and corresponding CD34⁻. Results are shown as the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

Figure 2: XPC expression throughout liquid culture of CD34⁺ cells

- A)** Western blot of CD34⁺ cells collected at different days during the culture was performed to reveal the expression of XPC.
- B)** Protein quantification of the western blot in (A) is represented by the ratio of XPC expression to actin expression.
- C)** Percentage of CD34⁺ cells were analyzed by FACS at different days of liquid culture.
- D)** Protein quantification of 125, 110 and kDa XPC bands in (A) is represented in form of ratio of XPC expression of each band to the total XPC expression at the different days of culture.
- E)** Western blot was performed on sorted CD34⁺ (95.2% ± 2.3) and CD34⁻ (100%) fractions originating from 11-days cultured CD34⁺ cells.
- F)** Protein quantification of the western blot in (E) is represented in form of ratio of normalized XPC/Actin expression in CD34⁺ cells and relative to CD34⁻ cells.

Results are shown as the mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Symbols: S: sample.

Figure 3: Effect of XPC on maintenance of CD34⁺ cells *in vitro*

- A) Representative western blot analysis of XPC expression of sorted GFP⁺ shXPC and shLuc transduced cells after seven days of liquid culture. Cells at day seven consisted of >96% CD34⁺ cells and 87% GFP⁺ cells.
- B) Protein quantification of XPC expression in WB of (A) and represented as normalized XPC/Actin expression and relative to NT. (n = 3 independent experiments).
- C) Follow-up of amount of GFP⁺ cells during liquid culture of CD34⁺ cells transduced with shLuc and shXPC vectors (n = 6).
- D) Graph representing the percentage of GFP at day 4 of culture of transduced CD34⁺ cells with shXPC or shLuc (control) determined by FACS from one side and the percentage of GFP⁺ colonies produced by shXPC and shLuc transduced CD34⁺ cells seeded in methylcellulose after 1 day of transduction (n=8).
- E) The percentage ratio of myeloid and erythroid GFP⁺ colonies has been determined by CFC assay relative to %GFP⁺ cells determined by FACS at day 4 of transduction in shLuc- and shXPC-transduced CD34⁺ seeded cells.

Results are shown as the mean \pm SEM. ** $p < 0.001$. Symbols: WB: western blot.

Figure 4: Effect of XPC on maintenance of CD34⁺ cells *in vivo*

- A) Schematic representation showing detailed description for the transplantation experiment conducted on NSG mice. 5 NSG mice were injected with shXPC-transduced CD34⁺ cells, 5 were injected with shLuc-transduced CD34⁺ cells serving as control and 5 other mice were injected with non-transduced CD34⁺ cells. The kinetics of human GFP⁺ cells was followed with time. Primary and secondary transplantations were performed.
- B) Fate of shXPC- or shCtrl- transduced CD34⁺ cells engrafted in NSG mice is assessed for by the follow-up of the kinetics of HuCD45⁺GFP⁺ cells amount at different time points (3 \pm 0.2, 6.2 \pm 0.17, 12.2 \pm 0.24 and 17.3 \pm 1.13 weeks) of engraftment by aspirating bone marrow from primary mice at each time point and analyzing it (n=5 experiments; 13 NT mice, 17 shCtrl mice, 16 shXPC mice).

- C) Histogram showing variation in ratio of % of HuCD45⁺GFP⁺ cells in shXPC relative to shCtrl in different organs (spleen, thymus, PB, BM) obtained from primary NSG recipient mice after 18 weeks of transplantation (n=5).
- D) Histogram showing variation in erythroid (CD36⁺GFP⁺, GPA⁺GFP⁺), myeloid (CD33⁺GFP⁺, CD11b⁺CD14⁺GFP⁺), and lymphoid (CD3⁺GFP⁺, CD19⁺GFP⁺) lymphoid lineage reconstitution between shXPC and shCtrl mice. Data is represented as ratio of percentage of each type of cell in shXPC relative to shCtrl after 18 weeks of transplantation (n=2 experiments, 7 shCtrl mice, 9 shXPC mice).
Results are shown as the mean ± SEM. *p < 0.05, ***p<0.001.

Figure 5: Effect of XPC on maintenance of stem cells and its reconstitution capacity

- A) Histogram showing the percentage of GFP⁺ cells relative to day 4 in different stem cell compartments 18 weeks after transplantation. Noteworthy, only FSC^{lo}SSC^{lo}huCD45⁺ cells were selected to gate for stem cells (7 shLuc mice, 5 shXPC mice). Only mice in which the more immature fraction (CD45RA⁻) provide enough events were selected (n = 3 experiments).
- B) Dot plot of the ratio of HuCD45⁺GFP⁺ cells in secondary mice relative to primary mice showing fate of HuCD45⁺GFP⁺ cells in secondary mice after 18 weeks of transplant (9 shCtrl mice, 6 shXPC mice).
- C) Histogram showing variation in the ratio of mean fluorescence of CellROX in GFP⁺ cells relative to GFP⁻ cells in Lin⁻CD34⁺ cells from bone marrow. The result represents the mean of 4 experiments (12 shLuc mice, 5 shXPC mice).
Results are shown as the mean ± SEM. *p < 0.05.

Supp 1: Investigation of XPC protein expression profile

- A) Western blot of keratinocyte cells from 8 patients analyzed for XPC expression profile using actin as loading control.
- B) Western blot of CD34⁺ cells isolated from 2 patients and analyzed simultaneously for XPC expression using both C-terminal anti-XPC antibody and N-terminal anti-XPC antibody. Keratinocyte was used as positive control.

Supp 2: Effect of XPC silencing on CD34⁺-derived cells

- A) Schematic representation of lentiviral construct used to inhibit XPC, Luc and RFP (control) expression is shown. The vector carry an internal cassette for the enhanced green fluorescent protein (GFP) driven by the EF-1α promoter and a cassette

containing the H1 promoter followed by a shRNA sequence targeting the XPC or Luc/RFP mRNA. Δ U3, R, and U5 are the LTR regions, with a deletion that includes the enhancer and the promoter from U3. cPPT is a nuclear import sequence.

- B)** Follow-up of proliferation of CD34⁺ cells transduced with shLuc and shXPC vectors (n = 3).
- C)** Follow-up of amount of CD34⁺ cells determined by FACS after liquid culture of cells transduced with shLuc and shXPC vectors (n = 4).

Figure 1:

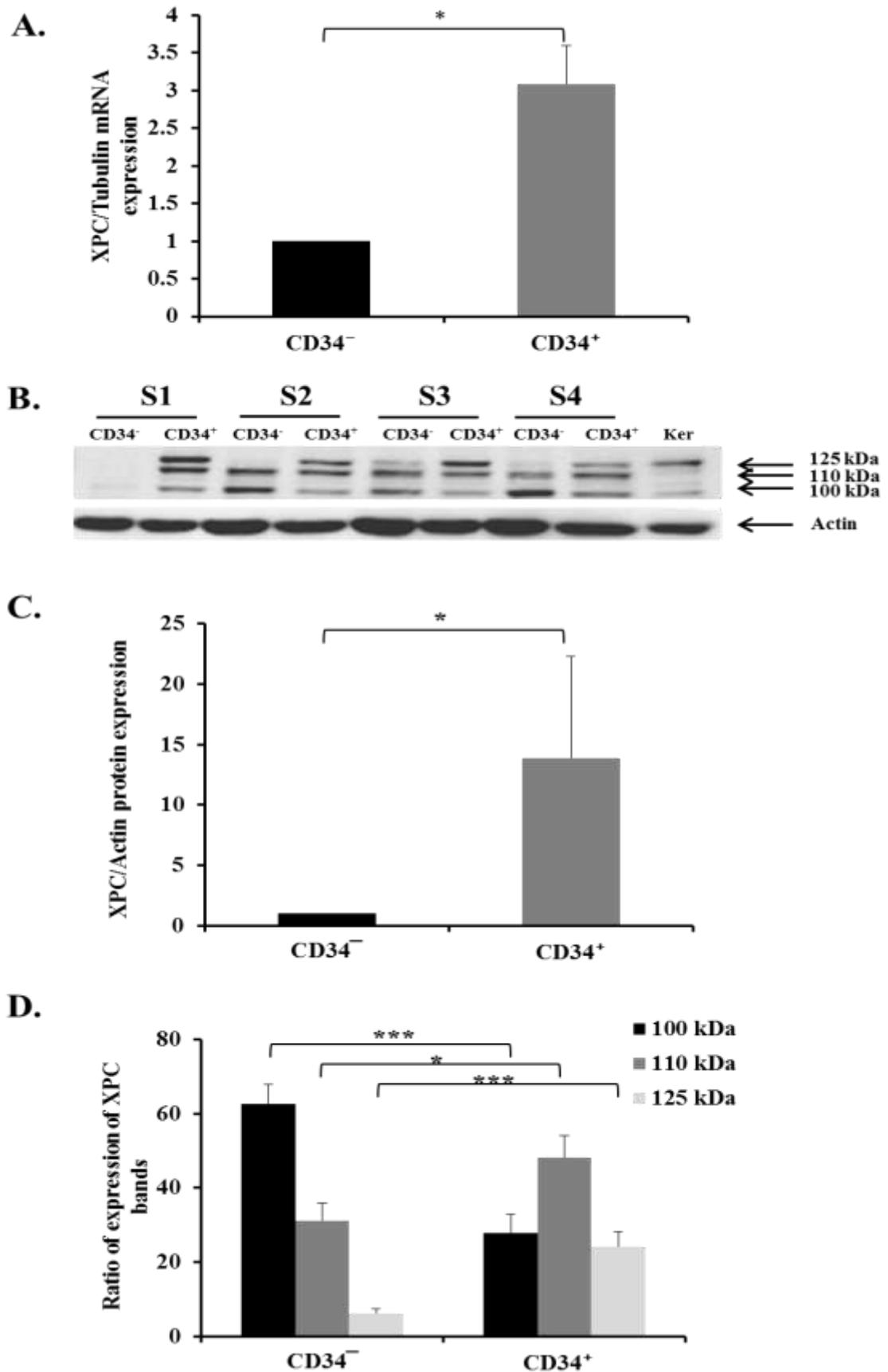


Figure 2:

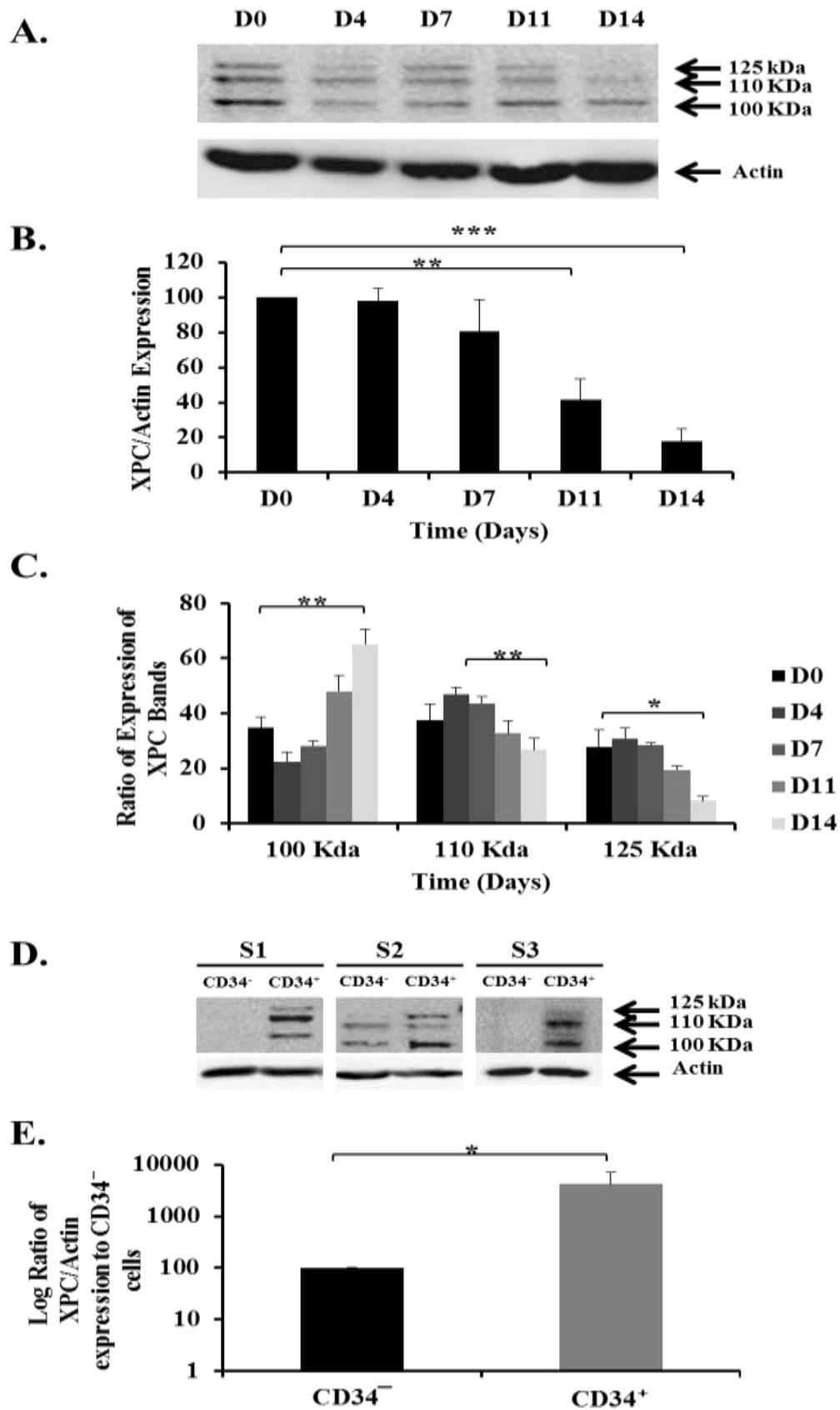


Figure 3:

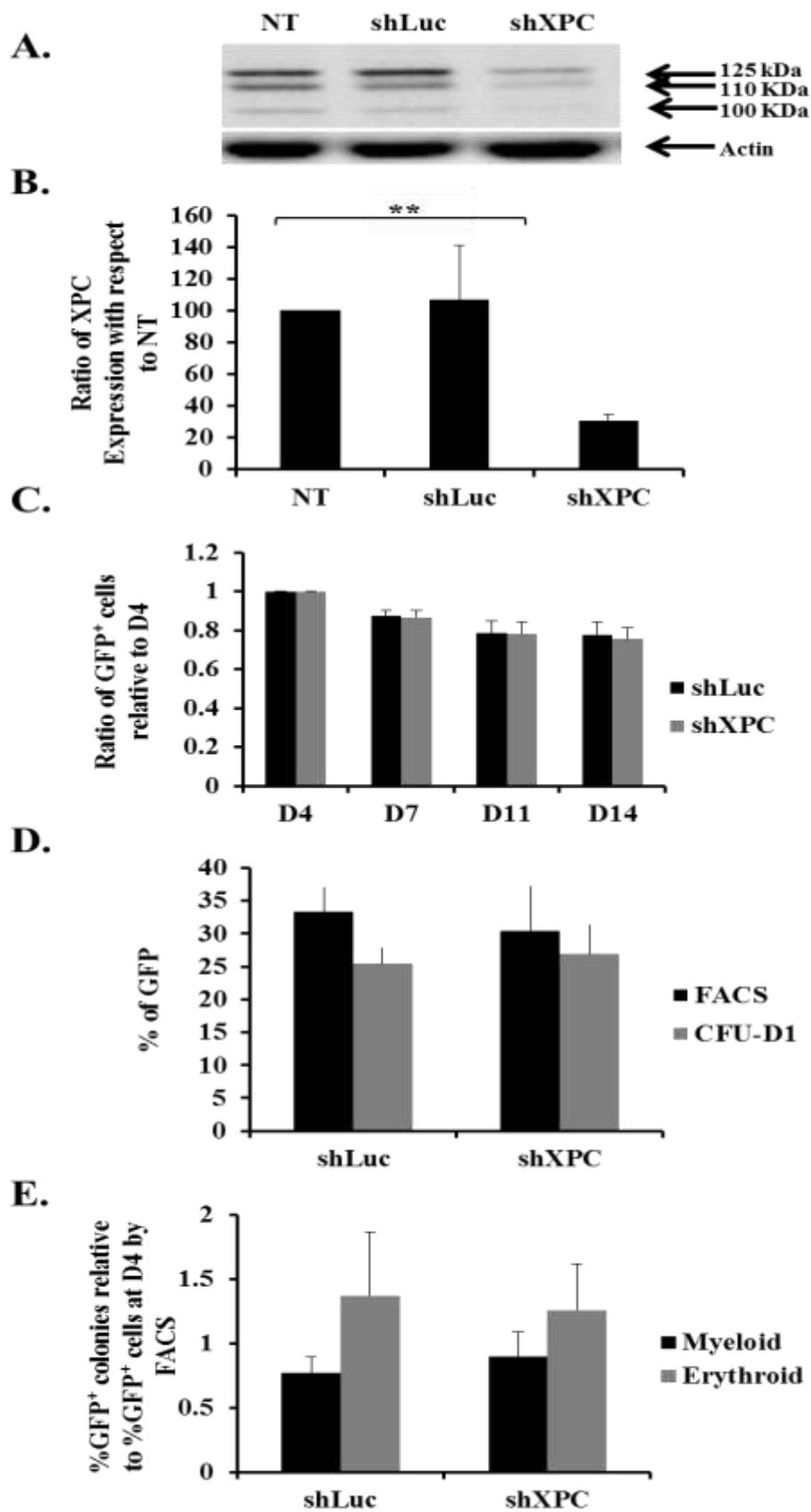


Figure 4:

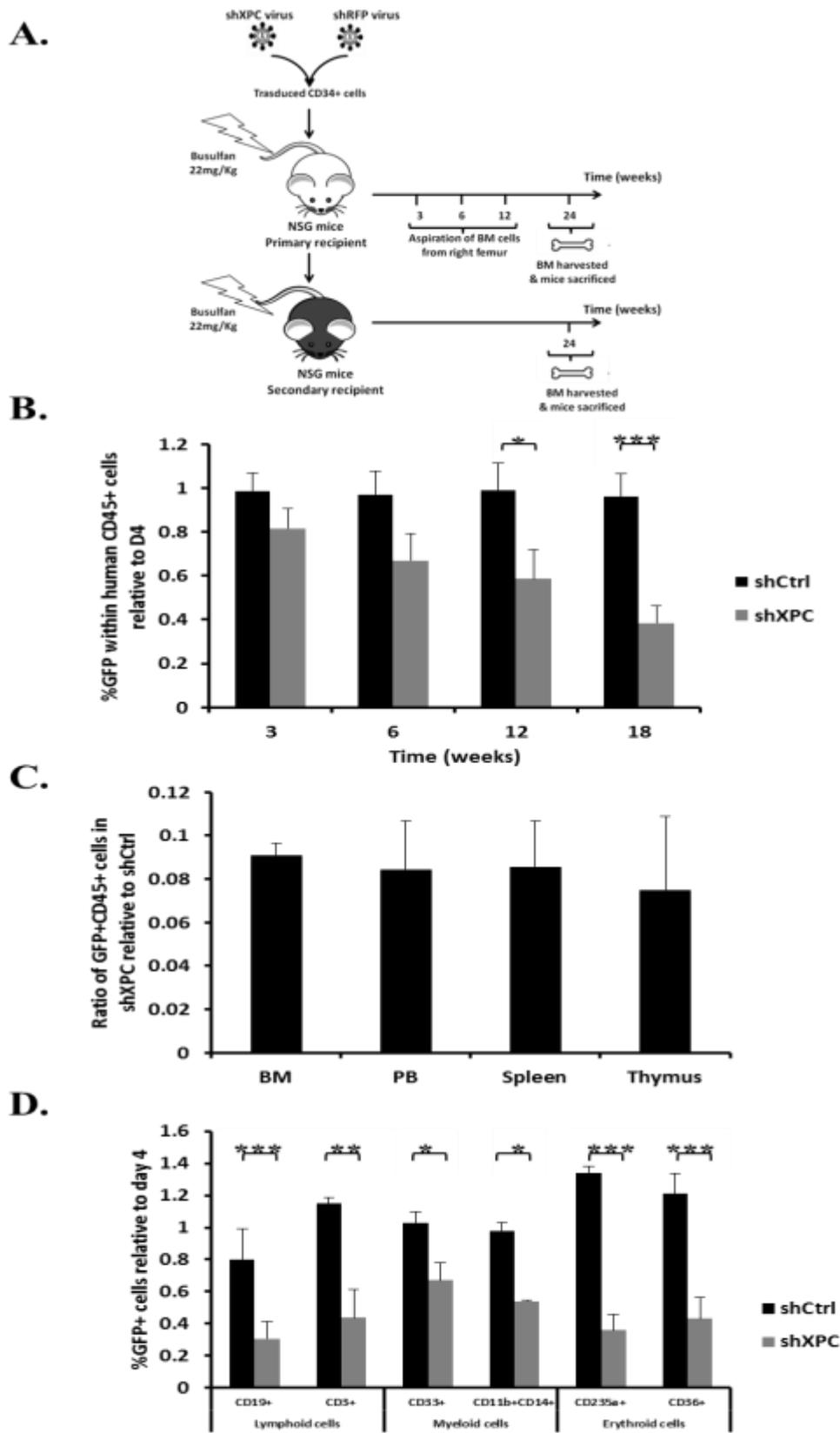
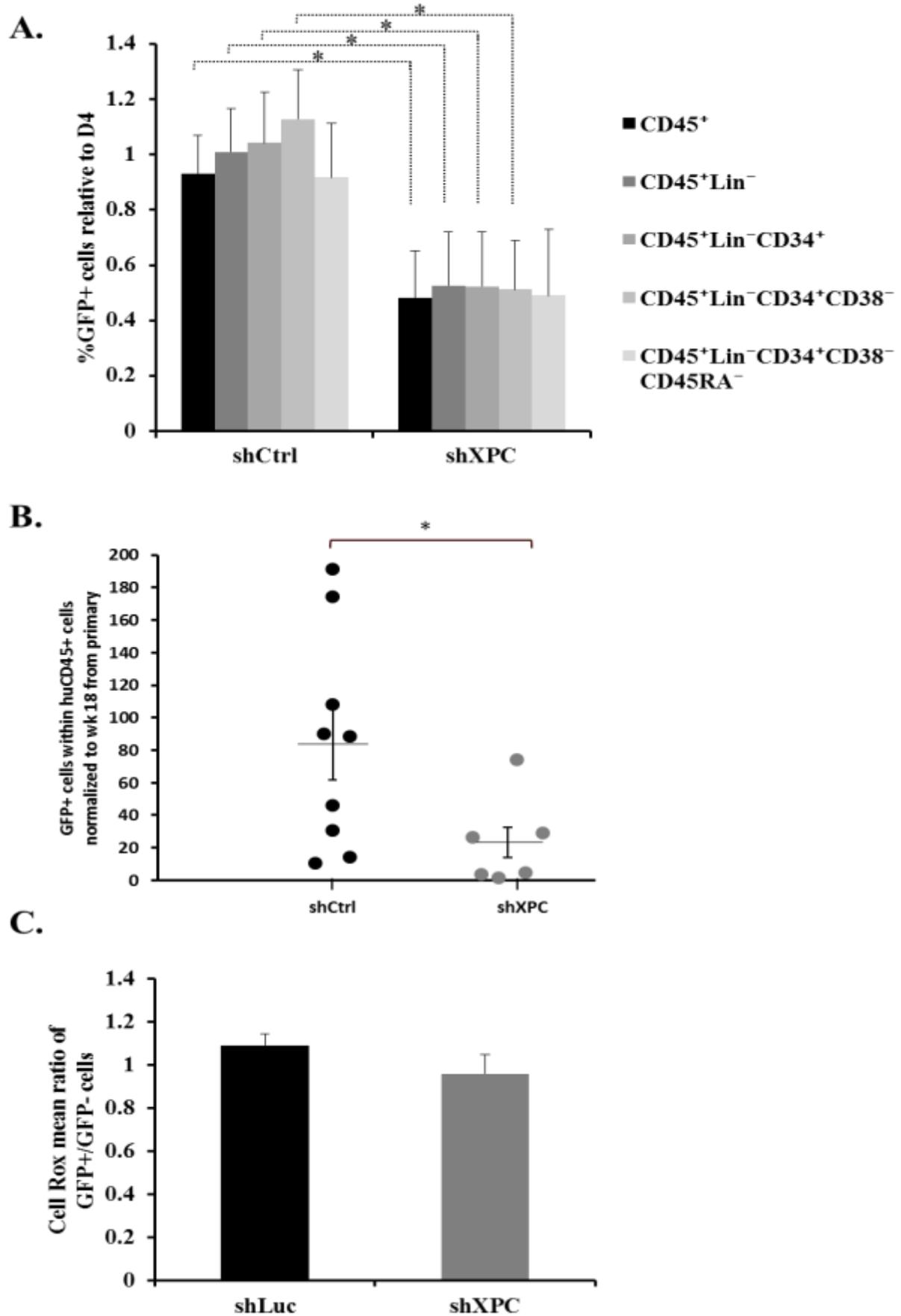
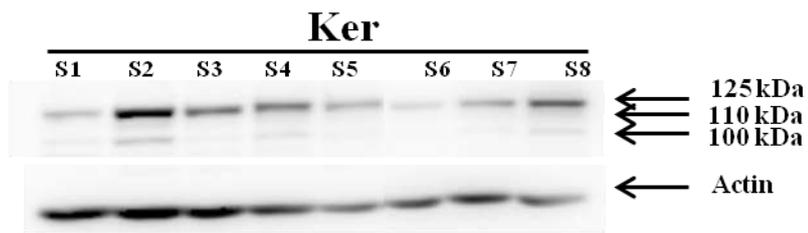


Figure 5:

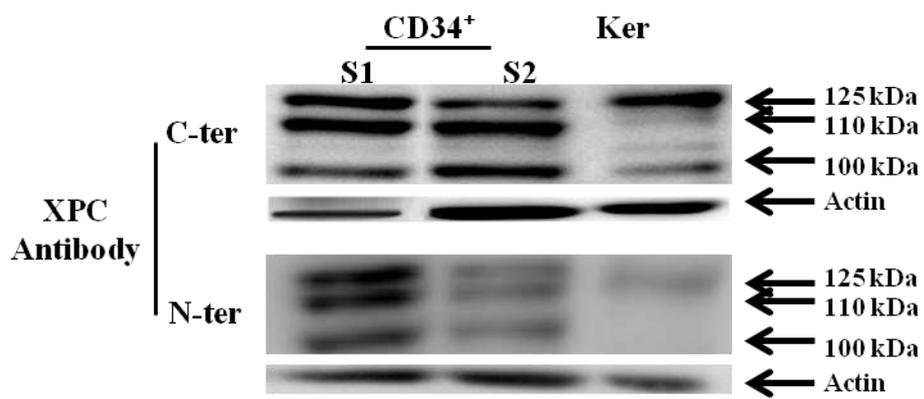


Supp 1:

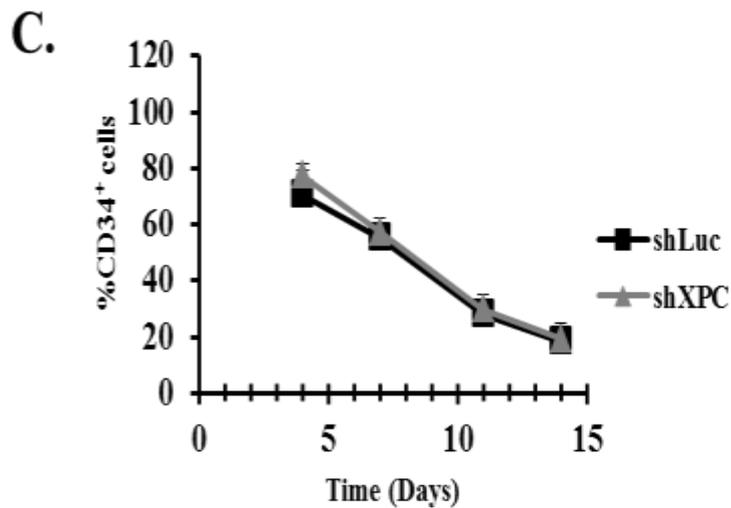
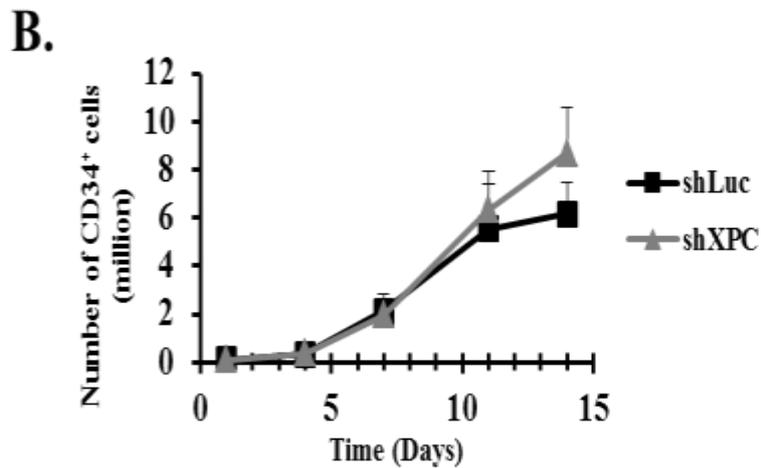
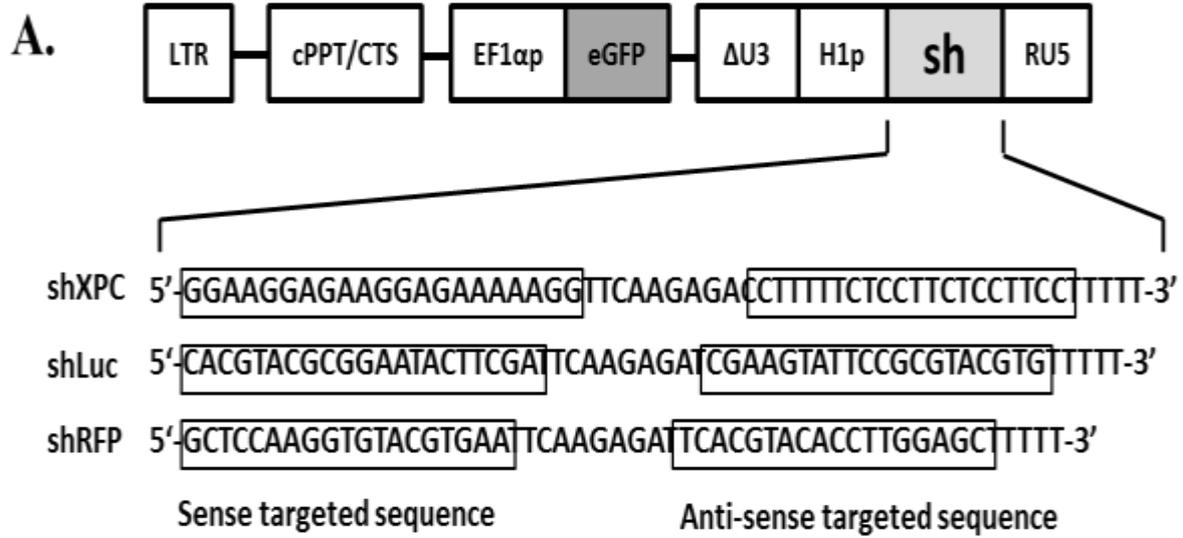
A.



B.



Supp 2:



References

- Aoki, Y., Sato, A., Mizutani, S., and Takagi, M. (2014). Hematopoietic myeloid cell differentiation diminishes nucleotide excision repair. *Int. J. Hematol.*
- Asai, T., Liu, Y., Bae, N., and Nimer, S.D. (2011). The p53 tumor suppressor protein regulates hematopoietic stem cell fate. *J. Cell. Physiol.* 226, 2215–2221.
- Barnes, D.J., and Melo, J.V. (2006). Primitive, quiescent and difficult to kill: the role of non-proliferating stem cells in chronic myeloid leukemia. *Cell Cycle Georget. Tex* 5, 2862–2866.
- Beerman, I., Seita, J., Inlay, M.A., Weissman, I.L., and Rossi, D.J. (2014). Quiescent Hematopoietic Stem Cells Accumulate DNA Damage during Aging that Is Repaired upon Entry into Cell Cycle. *Cell Stem Cell*.
- Bernstein, C., Bernstein, H., Payne, C.M., and Garewal, H. (2002). DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. *Mutat. Res.* 511, 145–178.
- Boisset, J.-C., and Robin, C. (2012). On the origin of hematopoietic stem cells: progress and controversy. *Stem Cell Res.* 8, 1–13.
- Bonnet, D. (2002). Haematopoietic stem cells. *J. Pathol.* 197, 430–440.
- Boon, W.-M., Beissbarth, T., Hyde, L., Smyth, G., Gunnensen, J., Denton, D.A., Scott, H., and Tan, S.-S. (2004). A comparative analysis of transcribed genes in the mouse hypothalamus and neocortex reveals chromosomal clustering. *Proc. Natl. Acad. Sci. U. S. A.* 101, 14972–14977.
- Bradford, G.B., Williams, B., Rossi, R., and Bertoncello, I. (1997). Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. *Exp. Hematol.* 25, 445–453.
- Bryder, D., Rossi, D.J., and Weissman, I.L. (2006). Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *Am. J. Pathol.* 169, 338–346.
- Chen, Z., Yang, J., Wang, G., Song, B., Li, J., and Xu, Z. (2007). Attenuated expression of xeroderma pigmentosum group C is associated with critical events in human bladder cancer carcinogenesis and progression. *Cancer Res.* 67, 4578–4585.
- Cleaver, J.E. (2005). Cancer in xeroderma pigmentosum and related disorders of DNA repair. *Nat. Rev. Cancer* 5, 564–573.
- D’Errico, M., Parlanti, E., Teson, M., de Jesus, B.M.B., Degan, P., Calcagnile, A., Jaruga, P., Bjørås, M., Crescenzi, M., Pedrini, A.M., et al. (2006). New functions of XPC in the protection of human skin cells from oxidative damage. *EMBO J.* 25, 4305–4315.
- Fischer, J.L., Kumar, M.A.S., Day, T.W., Hardy, T.M., Hamilton, S., Besch-Williford, C., Safa, A.R., Pollok, K.E., and Smith, M.L. (2009). The XPC gene markedly affects cell survival in mouse bone marrow. *Mutagenesis* 24, 309–316.
- Fong, Y.W., Inouye, C., Yamaguchi, T., Cattoglio, C., Grubisic, I., and Tjian, R. (2011). A DNA repair complex functions as an Oct4/Sox2 coactivator in embryonic stem cells. *Cell* 147, 120–131.

Fréchet, M., Warrick, E., Vioux, C., Chevallier, O., Spatz, A., Benhamou, S., Sarasin, A., Bernerd, F., and Magnaldo, T. (2008a). Overexpression of matrix metalloproteinase 1 in dermal fibroblasts from DNA repair-deficient/cancer-prone xeroderma pigmentosum group C patients. *Oncogene* 27, 5223–5232.

Fréchet, M., Warrick, E., Vioux, C., Chevallier, O., Spatz, A., Benhamou, S., Sarasin, A., Bernerd, F., and Magnaldo, T. (2008b). Overexpression of matrix metalloproteinase 1 in dermal fibroblasts from DNA repair-deficient/cancer-prone xeroderma pigmentosum group C patients. *Oncogene* 27, 5223–5232.

Hanawalt, P.C. (2002). Subpathways of nucleotide excision repair and their regulation. *Oncogene* 21, 8949–8956.

Hollander, M.C., Philburn, R.T., Patterson, A.D., Velasco-Miguel, S., Friedberg, E.C., Linnoila, R.I., and Fornace, A.J., Jr (2005). Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 102, 13200–13205.

Hsu, P., Hanawalt, P.C., and Nospikel, T. (2007). Nucleotide excision repair phenotype of human acute myeloid leukemia cell lines at various stages of differentiation. *Mutat. Res.* 614, 3–15.

Hu, Z., Wang, Y., Wang, X., Liang, G., Miao, X., Xu, Y., Tan, W., Wei, Q., Lin, D., and Shen, H. (2005). DNA repair gene XPC genotypes/haplotypes and risk of lung cancer in a Chinese population. *Int. J. Cancer J. Int. Cancer* 115, 478–483.

Hyka-Nospikel, N., Lemonidis, K., Lu, W.-T., and Nospikel, T. (2011). Circulating human B lymphocytes are deficient in nucleotide excision repair and accumulate mutations upon proliferation. *Blood* 117, 6277–6286.

Kondo, M., Wagers, A.J., Manz, M.G., Prohaska, S.S., Scherer, D.C., Beilhack, G.F., Shizuru, J.A., and Weissman, I.L. (2003). Biology of hematopoietic stem cells and progenitors: implications for clinical application. *Annu. Rev. Immunol.* 21, 759–806.

Kraemer, K.H., Lee, M.M., and Scotto, J. (1987). Xeroderma pigmentosum. Cutaneous, ocular, and neurologic abnormalities in 830 published cases. *Arch. Dermatol.* 123, 241–250.

De Laat, W.L., Jaspers, N.G., and Hoeijmakers, J.H. (1999). Molecular mechanism of nucleotide excision repair. *Genes Dev.* 13, 768–785.

Lee, K.-M., Choi, J.-Y., Kang, C., Kang, C.P., Park, S.K., Cho, H., Cho, D.-Y., Yoo, K.-Y., Noh, D.-Y., Ahn, S.-H., et al. (2005). Genetic polymorphisms of selected DNA repair genes, estrogen and progesterone receptor status, and breast cancer risk. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 11, 4620–4626.

Legerski, R., and Peterson, C. (1992). Expression cloning of a human DNA repair gene involved in xeroderma pigmentosum group C. *Nature* 359, 70–73.

Marín, M.S., López-Cima, M.F., García-Castro, L., Pascual, T., Marrón, M.G., and Tardón, A. (2004). Poly (AT) polymorphism in intron 11 of the XPC DNA repair gene enhances the risk of lung cancer. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* 13, 1788–1793.

Matsuoka, S., Ballif, B.A., Smogorzewska, A., McDonald, E.R., 3rd, Hurov, K.E., Luo, J., Bakalarski, C.E., Zhao, Z., Solimini, N., Lerenthal, Y., et al. (2007). ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316, 1160–1166.

Le May, N., Mota-Fernandes, D., Vélez-Cruz, R., Iltis, I., Biard, D., and Egly, J.M. (2010). NER Factors Are Recruited to Active Promoters and Facilitate Chromatin Modification for Transcription in the Absence of Exogenous Genotoxic Attack. *Mol. Cell* 38, 54–66.

Melis, J.P.M., Wijnhoven, S.W.P., Beems, R.B., Roodbergen, M., van den Berg, J., Moon, H., Friedberg, E., van der Horst, G.T.J., Hoeijmakers, J.H.J., Vijg, J., et al. (2008). Mouse Models for Xeroderma Pigmentosum Group A and Group C Show Divergent Cancer Phenotypes. *Cancer Res.* 68, 1347–1353.

Melis, J.P.M., Luijten, M., Mullenders, L.H.F., and van Steeg, H. (2011). The role of XPC: implications in cancer and oxidative DNA damage. *Mutat. Res.* 728, 107–117.

Melis, J.P.M., van Steeg, H., and Luijten, M. (2013). Oxidative DNA damage and nucleotide excision repair. *Antioxid. Redox Signal.* 18, 2409–2419.

Menoni, H., Hoeijmakers, J.H.J., and Vermeulen, W. (2012). Nucleotide excision repair-initiating proteins bind to oxidative DNA lesions in vivo. *J. Cell Biol.* 199, 1037–1046.

Miao, F., Bouziane, M., Dammann, R., Masutani, C., Hanaoka, F., Pfeifer, G., and O'Connor, T.R. (2000). 3-Methyladenine-DNA glycosylase (MPG protein) interacts with human RAD23 proteins. *J. Biol. Chem.* 275, 28433–28438.

Nguyen, T.-A., Slattery, S.D., Moon, S.-H., Darlington, Y.F., Lu, X., and Donehower, L.A. (2010). The oncogenic phosphatase WIP1 negatively regulates nucleotide excision repair. *DNA Repair* 9, 813–823.

Niedernhofer, L.J. (2008). DNA repair is crucial for maintaining hematopoietic stem cell function. *DNA Repair* 7, 523–529.

Nospikel, T., and Hanawalt, P.C. (2000). Terminally differentiated human neurons repair transcribed genes but display attenuated global DNA repair and modulation of repair gene expression. *Mol. Cell. Biol.* 20, 1562–1570.

Nospikel, T., and Hanawalt, P.C. (2002). DNA repair in terminally differentiated cells. *DNA Repair* 1, 59–75.

Piccoli, C., Ria, R., Scrima, R., Cela, O., D'Aprile, A., Boffoli, D., Falzetti, F., Tabilio, A., and Capitanio, N. (2005). Characterization of mitochondrial and extra-mitochondrial oxygen consuming reactions in human hematopoietic stem cells. Novel evidence of the occurrence of NAD(P)H oxidase activity. *J. Biol. Chem.* 280, 26467–26476.

Piccoli, C., D'Aprile, A., Ripoli, M., Scrima, R., Lecce, L., Boffoli, D., Tabilio, A., and Capitanio, N. (2007). Bone-marrow derived hematopoietic stem/progenitor cells express multiple isoforms of NADPH oxidase and produce constitutively reactive oxygen species. *Biochem. Biophys. Res. Commun.* 353, 965–972.

Reya, T., Morrison, S.J., Clarke, M.F., and Weissman, I.L. (2001). Stem cells, cancer, and cancer stem cells. *Nature* 414, 105–111.

Rezvani, H.R., Dedieu, S., North, S., Belloc, F., Rossignol, R., Letellier, T., de Verneuil, H., Taïeb, A., and Mazurier, F. (2007). Hypoxia-inducible factor-1alpha, a key factor in the keratinocyte response to UVB exposure. *J. Biol. Chem.* 282, 16413–16422.

Rezvani, H.R., Mahfouf, W., Ali, N., Chemin, C., Ged, C., Kim, A.L., de Verneuil, H., Taïeb, A., Bickers, D.R., and Mazurier, F. (2010). Hypoxia-inducible factor-1alpha regulates the expression of nucleotide excision repair proteins in keratinocytes. *Nucleic Acids Res.* 38, 797–809.

Rezvani, H.R., Rossignol, R., Ali, N., Benard, G., Tang, X., Yang, H.S., Jouary, T., de Verneuil, H., Taïeb, A., Kim, A.L., et al. (2011a). XPC silencing in normal human keratinocytes triggers metabolic alterations through NOX-1 activation-mediated reactive oxygen species. *Biochim. Biophys. Acta* 1807, 609–619.

Rezvani, H.R., Kim, A.L., Rossignol, R., Ali, N., Daly, M., Mahfouf, W., Bellance, N., Taïeb, A., de Verneuil, H., Mazurier, F., et al. (2011b). XPC silencing in normal human keratinocytes triggers metabolic alterations that drive the formation of squamous cell carcinomas. *J. Clin. Invest.* 121, 195–211.

Rezvani, H.R., Rossignol, R., Ali, N., Benard, G., Tang, X., Yang, H.S., Jouary, T., de Verneuil, H., Taïeb, A., Kim, A.L., et al. (2011c). XPC silencing in normal human keratinocytes triggers metabolic alterations through NOX-1 activation-mediated reactive oxygen species. *Biochim. Biophys. Acta* 1807, 609–619.

Rossi, D.J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J., and Weissman, I.L. (2007). Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* 447, 725–729.

Salob, S.P., Webb, D.K., and Atherton, D.J. (1992). A child with xeroderma pigmentosum and bone marrow failure. *Br. J. Dermatol.* 126, 372–374.

Schlessinger, D., and Van Zant, G. (2001). Does functional depletion of stem cells drive aging? *Mech. Ageing Dev.* 122, 1537–1553.

Seita, J., Rossi, D.J., and Weissman, I.L. (2010). Differential DNA damage response in stem and progenitor cells. *Cell Stem Cell* 7, 145–147.

Shimizu, Y., Iwai, S., Hanaoka, F., and Sugawara, K. (2003). Xeroderma pigmentosum group C protein interacts physically and functionally with thymine DNA glycosylase. *EMBO J.* 22, 164–173.

Shivji, M.K.K., Moggs, J.G., Kuraoka, I., and Wood, R.D. (2006). Assaying for the dual incisions of nucleotide excision repair using DNA with a lesion at a specific site. *Methods Mol. Biol. Clifton NJ* 314, 435–456.

Stout, G.J., Oosten, M. van, Acherrat, F.Z., Wit, J. de, Vermeij, W.P., Mullenders, L.H.F., Gruijl, F.R. de, and Backendorf, C. (2005). Selective DNA damage responses in murine Xpa^{-/-}, XPC^{-/-} and Csb^{-/-} keratinocyte cultures. *DNA Repair* 4, 1337–1344.

Strom, S.S., Estey, E., Outschoorn, U.M., and Garcia-Manero, G. (2010). Acute myeloid leukemia outcome: role of nucleotide excision repair polymorphisms in intermediate risk patients. *Leuk. Lymphoma* 51, 598–605.

Sugasawa, K. (2006). UV-induced ubiquitylation of XPC complex, the UV-DDB-ubiquitin ligase complex, and DNA repair. *J. Mol. Histol.* *37*, 189–202.

Sugasawa, K., Okuda, Y., Saijo, M., Nishi, R., Matsuda, N., Chu, G., Mori, T., Iwai, S., Tanaka, K., Tanaka, K., et al. (2005). UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. *Cell* *121*, 387–400.

Trego, K.S., and Turchi, J.J. (2006). Pre-steady-state binding of damaged DNA by XPC-hHR23B reveals a kinetic mechanism for damage discrimination. *Biochemistry (Mosc.)* *45*, 1961–1969.

Wang, G., Chuang, L., Zhang, X., Colton, S., Dombkowski, A., Reiners, J., Diakiw, A., and Xu, X.S. (2004). The initiative role of XPC protein in cisplatin DNA damaging treatment-mediated cell cycle regulation. *Nucleic Acids Res.* *32*, 2231–2240.

Wang, Q.-E., Zhu, Q., Wani, G., El-Mahdy, M.A., Li, J., and Wani, A.A. (2005). DNA repair factor XPC is modified by SUMO-1 and ubiquitin following UV irradiation. *Nucleic Acids Res.* *33*, 4023–4034.

Wang, Q.-E., Praetorius-Ibba, M., Zhu, Q., El-Mahdy, M.A., Wani, G., Zhao, Q., Qin, S., Patnaik, S., and Wani, A.A. (2007). Ubiquitylation-independent degradation of Xeroderma pigmentosum group C protein is required for efficient nucleotide excision repair. *Nucleic Acids Res.* *35*, 5338–5350.

Warrick, E., Garcia, M., Chagnoleau, C., Chevallier, O., Bergoglio, V., Sartori, D., Mavilio, F., Angulo, J.F., Avril, M.-F., Sarasin, A., et al. (2012). Preclinical corrective gene transfer in xeroderma pigmentosum human skin stem cells. *Mol. Ther. J. Am. Soc. Gene Ther.* *20*, 798–807.

Weissman, I.L. (2000). Stem cells: units of development, units of regeneration, and units in evolution. *Cell* *100*, 157–168.

Wu, X., Shell, S.M., Yang, Z., and Zou, Y. (2006). Phosphorylation of Nucleotide Excision Repair Factor Xeroderma Pigmentosum Group A by Ataxia Telangiectasia Mutated and Rad3-Related-Dependent Checkpoint Pathway Promotes Cell Survival in Response to UV Irradiation. *Cancer Res.* *66*, 2997–3005.

Van Zant, G., and Liang, Y. (2003). The role of stem cells in aging. *Exp. Hematol.* *31*, 659–672.

Zhou, S., Schuetz, J.D., Bunting, K.D., Colapietro, A.M., Sampath, J., Morris, J.J., Lagutina, I., Grosveld, G.C., Osawa, M., Nakauchi, H., et al. (2001). The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat. Med.* *7*, 1028–1034.

ANNEX 2

Review article in preparation

XPC beyond DNA repair: Novel functional insights

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Short title: **XPC crosstalk**

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Abstract

Xeroderma pigmentosum group C, XPC, has been widely known as a DNA damage recognition factor of bulky adducts and as an initiator of GG-NER pathway. Further studies have shown that XPC's role is not only limited to NER but also present several interactions with BER, HR, NHEJ and MMR pathways thereby influencing their proper functioning. Moreover, XPC is implicated in the DNA damage response (DDR) thereby participating in the cellular fate decision upon stress. Importantly, XPC has a proteolytic role manifested by its interaction with p53 and casp-2S and which determines the cellular outcome by its interaction with downstream proteins such as p21, ARF, p16 driving cells either to apoptosis, senescence, or tumor. Interestingly, novel clues indicate that XPC is involved in transcription regulation in the cell. Finally, XPC can be considered at the same time as a genomic caretaker and gatekeeper and can even be classified as a tumor suppressor. In this review, we shed light on XPC's synergism with the different molecular pathways in the cell.

1. Introduction

The cell represents a crowded factory of complex, tightly regulated, coordinated and overlapping molecular pathways that are governed by a DNA double helix that harbors the whole genetic material. The integrity of DNA double helix is fundamental for the survival of cells, tissues, organs and the organism. Throughout life, DNA integrity is continuously threatened by external and internal insults (Cadet et al., 2003; Friedberg et al., 2006; Mullaart et al., 1990) that can result in persistent DNA damage that will perturb cellular processes and eventually lead to mutations and disturbed homeostasis which may result in cancer and age-related diseases. Therefore, living cells have developed several ways to fix this DNA damage through repair pathways.

Several repair pathways present in living cells are in charge of repairing different types of DNA damage with varying fidelity and mutagenic consequences (Lombard et al., 2005). The repair pathways include NER (nucleotide excision repair), BER (base excision repair), MMR (mismatch repair), HR (homologous recombination), and NHEJ (non-homologous end-joining). Damaged nucleotides are mainly repaired with high fidelity by the error-free excision repair pathways (NER, BER), and reversal of DNA damage pathway (MMR), resulting into the correct nucleotide sequence and DNA structure. Single- and double-strand breaks, caused by fracture of the sugar-phosphate backbone, are mainly repaired by the error-free HR pathway or by the error-prone NHEJ pathway (Friedberg et al., 2006). Interestingly, XPC shows diverse interactions, either physical or regulatory, with the different repair pathways (**Table 1**).

2. XPC crossover with DNA repair pathways

2.1.XPC-NER interaction

The binding characteristics of XPC render it indispensable for the global genome (GG)-NER sub-pathway of NER. First, XPC protein contains several binding domains for DNA, HR23B, centrin2 and TFIIH. XPC itself has affinity to DNA and can initiate GG-NER, however, its stability requires assembly with centrin2 and HR23B proteins that inhibit polyubiquitinylation of XPC and its degradation by the 26S proteasome (Araki et al., 2001; Nishi et al., 2005). The above assembled complex serves to stabilize the low-affinity XPA and RPA proteins that subsequently bind at the damaged site. Second, XPC is responsible for the recognition of various helix-distorting base lesions that do not share a common chemical structure. Biochemical studies have revealed that XPC recognizes a specific secondary DNA structure rather than the lesions themselves (Sugasawa et al., 2001, 2002). In fact, it has been suggested

that XPC binds opposite to the lesion site and flips the damaged bases out of the helix structure, providing an explanation for the broad specificity of GG-NER damage recognition (Min and Pavletich, 2007). Third, XPC appears to scan the DNA for distortions by migrating over the DNA, together with DDB1 and DDB2, binding and dissociating from the double helix repeatedly. When XPC encounters a lesion the protein changes its conformation and aromatic amino acid residues of XPC stack with unpaired nucleotides opposite the lesion (Hoogstraten et al., 2008). Fourth, the binding affinity of XPC to the DNA correlates with the extent of helical distortion since highly distorting lesions provide sufficient opening of the DNA strands so that XPC can be dispensed within cell-free systems of NER (Sancar, 1996; Wood, 1997). For example, XPC binds with higher specificity to UV-induced 6-4PP adducts, that substantially distort the DNA structure by approximately 44° towards the major groove (Kim et al., 1995), than to UV-induced CPDs, which only cause a 30° bending in DNA (Park et al., 2002) and which require the assistance of DDB protein (Tang et al., 2000). Fifth, XPC binding appears to be asymmetric and can occur in a productive or non-productive binding mode. In the productive binding mode, XPC is bound to the undamaged strand; this leads to the $5' \rightarrow 3'$ translocation and strand opening catalyzed by XPD in TFIIH that yields the dual incisions. In the non-productive binding mode, XPC is bound to the damaged strand and does not lead to lesion excision (Sugasawa et al., 2002, 2009). Sixth, it has been shown that more than one XPC molecule could be bound at the damaged site of DNA (Lee et al., 2014). Nevertheless, the role of XPC in DNA lesion recognition has been shown recently to include a dark side (Puumalainen et al., 2014). In fact, the beneficial DNA repair response triggered by XPC and DDB2 depends critically on a dynamic spatio-temporal regulation of their homeostasis mediated by ubiquitin-dependent p97 segregase complex. Recruitment of p97 to UV lesions is primarily dependent on DDB2 and to a lesser degree on XPC. Failure to remove ubiquitylated XPC and DDB2 due to p97 deficiency leads to impaired DNA excision repair and provokes formation of chromosomal aberrations. Thus, XPC functions as a double-edged sword since it is essential to trigger a beneficial DNA repair activity but may become detrimental to the genome, if allowed to accumulate in damaged chromatin without control by the p97 segregase (Puumalainen et al., 2014).

Interestingly, XPC is subject to several posttranslational modifications which are likely to regulate its role in NER. XPC has been found to be phosphorylated by ATM and ATR (Matsuoka et al., 2007; Wu et al., 2006). On the other hand, WIP1, a serine/threonine wild-type p53-induced phosphatase 1, specifically dephosphorylates XPC at Ser 892 after repairing DNA damage leading to its inactivation and suppression of NER (Nguyen et al., 2010). In addition, XPC has been found to be ubiquitylated by DDB2, an assembly protein of XPC

complex that forms a part of E3 ubiquitin ligase known as DDB2-DDB1-Cul4A-RBX1 complex. The cullin subunit serves as a scaffold to assemble on one side an E2-binding subunit (RBX1/ROC1 or RBX2) and on the other side a target-recognition complex, consisting of DDB1 as an adaptor subunit and DDB2/XPE as a target receptor. Shortly after exposure to UV (30min), COP9 signalosome (CSN), which inhibits ubiquitin ligase activity by de-neddylating CUL4 and trimming the ubiquitin chains, detaches from E3 complex, CUL4 is then neddylated which stimulates the ubiquitin ligase activity of the complex (Groisman et al., 2003). XPC is then polyubiquitinated by the ROC1-CUL4A-DDB1-DDB2 complex, causing an increase in the affinity of XPC for DNA, damaged or not. Then DDB2 and CUL4A self-ubiquitinate resulting in a drastic decrease in affinity of the E3 complex for damaged DNA leading to its dissociation and degradation by a proteasome-dependent manner (Sugasawa, 2006b; Sugasawa et al., 2005). Ubiquitinylation of XPC does not target it towards degradation; instead, XPC is deubiquitinated and returned to its original state by 4h after irradiation. It is worth noting that XPC degradation observed after UV irradiation was not triggered by XPC polyubiquitinylation, but rather by direct interaction with the proteasome. On the contrary, XPC ubiquitinylation played a protective role against proteasomal degradation (Wang et al., 2007). A further level of control was revealed by the recent discovery that XPC enhances E3 activity of the DDB complex (Takedachi et al., 2010). HHR23B, that comprises ubiquitin-like domain and two ubiquitin-binding domains in addition to XPC binding domain (Dantuma et al., 2009), is thought to protect XPC against degradation during ubiquitinylation (Nospikel, 2011). XPC has also been shown to interact with OTUD4 deubiquitinase in human cells where OTUD4 knockdown affected the levels of XPC suggesting that OTUD4 is involved in XPC recycling by cleaving the ubiquitin moiety (Lubin et al., 2014). Finally, XPC stability requires assembly with centrin2 and HR23B proteins which inhibit polyubiquitinylation of XPC and its degradation by the 26S proteasome (Araki et al., 2001; Nishi et al., 2005). In addition, upon UV irradiation of normal human fibroblasts, XPC protein undergoes covalent post-translational modifications including sumoylation by SUMO-1, believed to stabilize XPC protein, and ubiquitinylation by ubiquitin (Wang et al., 2005). These modifications require the function of XPA, DDB2 and the ubiquitin-proteasome system. Recently, Poulsen et al. has shown that human RNF111/Arkadia ubiquitin ligase is a SUMO-targeted ubiquitin ligases (STUbLs) that selectively recognizes SUMOylated target proteins through SUMO-interacting motifs (SIMs), promoting their nonproteolytic ubiquitinylation. Interestingly, RNF111-catalyzed DNA damage-induced ubiquitinylation of SUMOylated XPC promoted ubiquitinylation of

SUMOylated XPC and served in regulating the recruitment of XPC to UV-damaged DNA, thereby facilitating NER (Poulsen et al., 2013) (**Figure 1**).

2.2.XPC-BER interaction

Strong lines of evidence indicate that XPC is implicated in BER pathway. First, *Xpc* and *Xpc* deficient mice show differences in oxidative DNA damage sensitivity indicating that XPC has additional functions outside of NER. Since BER is the primary pathway for repairing oxidative lesions and XPC defect leads to increased sensitivity towards oxidative DNA damage, it is thought that XPC is implicated in BER pathway (Melis et al., 2008a). In addition, mouse embryonic fibroblasts (MEFs) derived from *Xpc*^{-/-} mice and exposed to oxygen showed higher sensitivity, in terms of survival and mutation accumulation, than MEFs derived from *Xpc*^{-/-} or wild type mice (Melisa et al., 2008a). Moreover, *Xpc*^{-/-} mice develop more spontaneous mutations and more tumors upon 2-acetylaminofluorene treatment, in comparison to wild-type or *Xpa*^{-/-} mice, (Hoogervorst et al., 2005; Wijnhoven et al., 2000). Second, Meira et al. presented an evidence for XPC interaction with Apex (*APE* or *Ref-1* in humans), which is a critical component of BER pathway that codes for apurinic/apyrimidinic (AP) endonuclease function in mouse cells. Indeed, the effect of *Apex* heterozygous mutation is dependent on defective NER since it only manifests when *XPC* is mutated. *Apex* heterozygous animals are not more cancer-prone than wild-type animals and the double heterozygous mutant *XPC*^{+/-}*Apex*^{+/-} shows the same skin cancer incidence as the *XPC*^{+/-} mutant alone. On the other hand, combination of XPC mutation with *Apex* heterozygosity increases the predisposition to skin cancer by decreasing its latency time suggesting that XPC complements the action of *Apex* (Meira et al., 2001a). Third, XPC has been shown to interact with 3-methyladenine DNA glycosylase (TAG) (Miao et al., 2000) in a similar manner that hHR23B factor does (Kraemer et al., 1992). Fourth, XPC was shown to be implicated in repair of oxidative DNA damage by its interaction with thymine DNA glycosylase (TDG), which is an initiator of BER and that responds to G/T mismatches formed from the deamination of 5-methylcytosines. Indeed, XPC-RAD23B was shown to form a complex with TDG-bound DNA and to stimulate TDG activity (Shimizu et al., 2003a). XPC is also able to interact with single-strand selective monofunctional uracil DNA glycosylase (SMUG1) which is a glycosylase that removes uracil from single- and double-stranded DNA in nuclear chromatin, thus contributing to BER (Shimizu et al., 2003a). Fifth, XPC has been shown to interact with 8-oxoguanine DNA glycosylase (D'Errico et al., 2006). It has been postulated that the XPC-hHR23B complex acts as a co-factor in the base excision repair of 8-hydroxyguanosine products, by stimulating the activity of the BER DNA glycosylase OGG1. D'Errico and

colleagues have proposed a mechanism in which XPC–HR23B might bend DNA at sites of damage and, thus, facilitate loading and turnover of DNA glycosylases by direct protein–protein interaction or by competition with the DNA substrate. Moreover, they showed that XPC protects human skin cells from the killing effects of oxidants such as potassium bromate or those induced by X-rays (D’Errico et al., 2006). Sixth, analysis of the biochemical properties behind mutations in the *XPC* gene found in XP patients demonstrated a direct interaction between the N-terminal part that encompasses the P334 surrounding region of XPC and OGG1. The XPC/P334H mutation weakens the interaction with OGG1, resulting in a decreased capacity to regulate the glycosylase activity (Bernardes de Jesus et al., 2008). Seventh, fibroblasts from different XPC patients also showed impairment in BER of oxidative DNA damage induced by methylene blue plus visible light (Kassam and Rainbow, 2007). Another study showed higher ROS levels in XPC cells, compared to control fibroblasts (Fréchet et al., 2008a). In addition, Liu et al. witnessed a slight increase in PARP-1 in siXPC cells exposed or not to arsenic trioxide (ATO), a DNA damaging agent that exerts its cytotoxicity by ROS generation and inhibition of DNA repair. Moreover, exposure of human glioma cells to ATO induced the expression of XPC, but not XPA. It’s important to note that silencing of XPC increased the susceptibility to ATO-induced cell death as well as increased senescence and autophagy but did not interfere with repair of ATO-induced DNA damage. However, treatment with N-acetylcysteine (NAC) suppressed the enhanced superoxide production and autophagy by ATO in siXPC cells (Liu et al., 2010). Furthermore, a study performed by Rezvani et al. on normal human keratinocytes silenced for XPC revealed an increased intracellular ROS levels, genomic and mtDNA oxidation and altered metabolic alterations due to disturbed redox homeostasis that in consequence lead to mutation accumulation and tumorigenesis (Rezvani et al., 2011a). It was also demonstrated that silencing of XPC in normal human keratinocytes appeared to activate NADPH oxidase-1 (NOX-1) which upregulates ROS production. Eighth, XPC was found to have a role in protection of cells against oxidative DNA damage where XPC was strongly and very rapidly recruited to sites of oxidative DNA lesions in living cells (Menoni et al., 2012). It also provided the first *in vivo* indication of the involvement of XPC in the repair of oxidative DNA lesions independent of the remainder NER reaction. XPC was found to recognize 8-oxoG (8-oxo-7,8-dihydroguanine) that is known to destabilize DNA helix by inducing changes in hydrophilicity of bases and impacting on major groove cation binding which renders this lesion a directly XPC recognizable target (Singh et al., 2011). XPC was revealed to accumulate more abundantly in DNA-dense heterochromatic subnuclear areas. Indeed, it was apparent that XPC follows a typical chromatin/DNA distribution pattern and XPC recruitment

to damaged DNA seems independent of transcriptional status and the lower amount of recruited protein in nucleoli is in accordance with relatively low DNA content in it (Menoni et al., 2012). Ninth, an indirect link between XPC and PARP-1 (polyADP-ribose polymerase-1) has been found, mediated through DDB2. In fact, PARP1 is not directly involved in BER but its inhibition delays repair of SSBs by BER by trapping PARP on the SSB intermediate formed during BER (Strom et al., 2011). Indeed, PARP-1 collaborates with DDB2 to increase the efficiency of the lesion recognition step of GG-NER where DDB2 associates with PARP-1 in the vicinity of UV-damaged chromatin, stimulates its catalytic activity, and is modified by pADPr. Deficiency/inhibition of PARP-1 suppresses UV association of DDB2 with XPC, inhibits slow migrating ubiquitinated XPC, known to be elevated in response to UV, and reduces colocalization with XPC. PARP inhibition disrupted interaction of XPC with DDB2 and decreased its stability at lesion site (Robu et al., 2013). Finally, although non-essential for BER, XPC widely contributes to the effectiveness of this repair pathway by possibly recognizing or enabling recognition of oxidative lesions through signaling and regulatory functions.

2.3.XPC-MMR Interaction

Several evidences exist for XPC interaction with MMR pathway. Indeed, MutS β and XPC-RAD23B are involved in the recognition of interstrand cross links (ICLs), the most cytotoxic types of DNA damage induced by psoralen, *in vitro* and *in vivo*. These proteins may participate cooperatively or independently in ICL repair, depending on their local concentrations. At low protein concentrations, MutS β and XPC-RAD23B bind the psoralen ICLs independently. However, increasing the concentrations of MutS β and XPC-RAD23B triggered the formation of a higher-order complex containing the psoralen ICLs bound to both protein complexes (Zhao et al., 2009). It was previously demonstrated that XPC interact with Msh2 using UV-B induced skin cancer. In fact, *Msh2*^{-/-} single mutant animals manifested increased skin cancer predisposition when compared to wild-type controls. In addition, combining the *XPC* and *Msh2* mutations in *XPC*^{-/-}*Msh2*^{-/-} double mutant mice resulted in a significant acceleration of the incidence of tumors (Meira et al., 2001a). Finally, it has been revealed that transcription of mLh1 and msh2 increased in XPC-defective cells upon cisplatin treatment (Wang et al., 2004).

2.4.XPC-HR interaction

Several lines of evidence show that XPC influence HR mediated DNA repair pathway in different ways. Arlett et al. described an unusual radiosensitive XPC patient (Arlett et al.,

2006). In addition, XPC association with the centrosomal protein centrin 2 was suggested to couple NER to cell division (Araki et al., 2001). Interestingly, it was found that reduced mRNA level of centrin 2 in a plant mutant is associated with UVC sensitivity, decreased NER efficiency, and increased HR (Molinier et al., 2004). For instance, upon cisplatin treatment, an interstrand crosslinking agent used in tumor therapy, XPC defect affected transcription responses of many genes, including DNA DSB repair genes (Wang et al., 2004). Also, in cisplatin-treated cells, XPC interacted physically with ATM protein ATM, a DNA damage sensor involved in HR pathway, and activated it enabling its association with genomic DNA and thereby preventing cisplatin-induced apoptosis (Colton et al., 2006). Moreover, upon UV radiation, SNF5, a member of SNF5/INI1 chromatin remodeling component, colocalizes and interacts with XPC which facilitates the access of ATM to the damaged site and thereby promotes NER (Ray et al., 2009). Furthermore, ATM protein and Rad3 related protein (ATR) appeared to be required for GG-NER, exclusively in S phase of human cells (Auclair et al., 2008, 2009). Ray et al. showed that, upon UV exposure, cells defective in XPC or DDB2 manifested a marked decrease in BRCA1 and Rad51 recruitment to the damage site (Ray et al., 2013). In fact, XPC was shown to be implicated in signaling of ATM and ATR protein kinases that are key regulators in DNA damage response, especially DSBs, by interacting with cell cycle checkpoints leading to G1 or G2 arrest or S phase delay. Indeed, DNA damage caused by UV exposure is regulated by XPC and DDB2 that are responsible for the recruitment of ATM and ATR kinases by physical interaction to DNA damage sites. The activated ATM and ATR kinases subsequently phosphorylate their substrates checkpoint kinases Chk1 and Chk2, H2AX, and BRCA1. On the other hand, ATR- and ATM-deficiency failed to affect the recruitment of DDB2 and XPC to the damage site, and therefore did not influence the NER efficiency. DDB2 and XPC play a definitive role in regulating ATR-Chk1-BRCA1 and ATM-Chk2-BRCA1 downstream signaling in realm of UV damage response (Ray et al., 2013). XPC appears also to influence γ H2AX levels (Hanasoge and Ljungman, 2007), which is created by the phosphorylation of the histone variant H2AX on serine 139, and which is characterized as a very early event following induction of certain types of DNA damage such as DSBs. Formation of γ H2AX is mainly known to be induced by IR (Burma et al., 2001) but it was later found to be produced constitutively in the cells due to oxidative stress (Tanaka et al., 2006) in ATM-dependent manner. It is known that γ H2AX is formed following UV irradiation in S phase-dependent DSB formation manner where the UV-induced bulky adducts collide with replication forks leading to their stalling and eventual fork collapse, provoking DSBs formation which will in turn trigger γ H2AX formation (Ward et al., 2004). On the other hand, another study revealed that H2AX phosphorylation occurs in all

phases of cell cycle through a mechanism that is replication-independent triggered by DNA repair intermediates and mediated by ATR kinase (Matsumoto et al., 2007). Interestingly, GGR-deficient XPC cells show much lower levels of γ H2AX than TCR-deficient CSB cells (Hanasoge and Ljungman, 2007). Recently, a novel study has provided a clear evidence for XPC's influence on ATM and ATR kinases, their substrates and γ H2AX formation by examining the role of XPC in the DNA damage response (DDR) of human melanocytes. Upon UV-induced DNA damage, α -melanocortin (α -MSH) activates melanocortin 1 receptor (MC1R) that will stimulate eumelanin synthesis but also will upregulate XPC, which enhances UV-induced phosphorylation of the DNA damage sensors ATR and ATM (Ray et al., 2013; Smith et al., 2010; Yajima et al., 2009) and their respective substrates checkpoint kinases 1 and 2 (Chk1 and Chk2), involved in cell cycle arrest, and increased γ H2AX formation (Barnes et al., 2010; Kinner et al., 2008; Mah et al., 2010). The upregulated XPC, ATM and ATR will also increase the UV-induced activation of p53 (Kadekaro et al., 2012) and p53-dependent phosphatase Wip1, which leads to dephosphorylation of p-H2AX (γ H2AX) and termination of the DDR once the conditions for restoration of homeostasis, genomic stability and melanocyte survival are set in place (Swope et al., 2014).

2.5.XPC-NHEJ interaction

Despras et al. presented strong evidence for the interaction between XPC and NHEJ pathway. Long-term silencing of XPC gene in HeLa cell line revealed an enhanced sensitivity to etoposide, which is a topoisomerase II inhibitor that creates DSBs. On the other hand, this effect was not seen in XPA^{KD} cells, an intolerance toward acute doses of γ -rays and most interestingly an altered spectrum of NHEJ products. Upon treatment with calicheamicin, a potent and very specific DSB inducer, the association of phosphorylated XRCC4 and ligase IV occurred at lower doses in XPC^{KD} cells and also in DNA-PK_{CS}^{KD} cells. In addition, treatment with highly specific DSB inducers lead to dose-dependent degradation of XPC, which was believed to be a step of the cellular response to DSBs in HeLa cells (Despras et al., 2007). Furthermore, Rezvani et al. showed that although silencing of XPC in human keratinocytes lead to apoptosis of the majority of cells, some cells escaped the apoptotic pathway and activated the error-prone NHEJ pathway. In comparison to control cells, XPC deficient cells showed an increase in RNA transcript levels of DNA-PKcs and Ku70/XRCC6 and a similar increase in the protein levels of DNA-PKcs, Ku70/XRCC6, XRCC4, and DNA ligase IV. Moreover, XPC deficient cells showed an increase in DNA-PK kinase activity and a reduction in correct end joining. At the molecular level, activation of DNA-PK in XPC-silenced cells lead to activation of AKT by its phosphorylation at Ser 473 which in turn

activated NOX1, thereby leading to elevated ROS production and accumulation of specific deletions in mitochondrial DNA over time. Moreover, subcutaneous injection of XPC deficient keratinocytes into immunodeficient mice lead to formation of squamous cell carcinoma which was blocked by NOX1 or AKT1 inhibition (Rezvani et al., 2011b).

3. XPC in DNA-damage response

The DNA-damage response (DDR) represent an evolutionary conserved group of signaling pathways turned on directly after sensing any occurrence of DNA lesion, irrespective of its type and the repair mechanism initiated. DDR is continuously present to insure genomic stability of cells and include cell cycle checkpoint activation, apoptosis and senescence (Bartek and Lukas, 2007). Multiple lines of evidence show that XPC influences cell-fate decision by its interaction with molecular players of the DDR response.

3.1.XPC-p53 interaction: A feedback loop

At the molecular level, the most prominent player in DDR is p53 which is phosphorylated, stabilized and accumulates in the nucleus, upon DNA damage. p53 is a well-known tumor suppressor and a central regulator of DDR that triggers an array of events in order to restore genomic integrity, including transcription induction, cell cycle arrest, and apoptosis (Brooks and Gu, 2011; Wade et al., 2010). Therefore, different outcomes are generated depending upon the extent of DNA damage, rapidity of its repair, the type of cell undergoing DNA damage and its cell cycle stage, strength and duration of p53 activation and its transactivated genes. The constitutive level of XPC expression is controlled by p53, and can be induced by UV-light irradiation, enhancing GGR (Ford and Hanawalt, 1997). Hence, compromised p53 function might reduce GG-NER activity, thereby facilitating accumulation of mutations and carcinogenesis. Additionally, XPC deficiency is strongly correlated with p53 mutations and malignancy in bladder tumors (Chen et al., 2007). Interestingly, a mutational hotspot at a nondipyrimidinic CpG site in codon 122 was detected in Trp53 of UVB-induced skin tumors in *XPC*^{-/-} mice that cannot be detected in the absence of XPA or Cockayne syndrome A (Nahari et al., 2004; Reis et al., 2000).

XPC interaction with p53 was demonstrated in mice where a combination of *XPC* mutation and *Trp53* heterozygosity mutation enhanced skin cancer predisposition (Meira et al., 2001a). In fact, p53 is required for efficient NER of UV-induced DNA lesions. Indeed, p53 mediates the GGR through the coordinate transcriptional control of a set of DNA damage recognition NER factors among which is XPC. Adimoolam and Ford showed that XPC is a p53-regulated

gene being positively regulated transcriptionally by p53 after DNA damage. Interestingly, XPC promoter contains putative p53 response element identified by sequencing of the human XPC gene. In human, XPC expression at both RNA and protein level is induced by UV in a time- and dose-dependent manner in WI38 fibroblasts and HCT116 colorectal cancer cells, which have a wild type p53. On the other hand, no significant induction of XPC was observed in p53-deficient counterparts to these cells. In addition, regulated expression of wild-type p53 in p53 null Li-Fraumeni syndrome human fibroblasts significantly augmented the expression of XPC protein (Adimoolam and Ford, 2002). Moreover, the role of XPC protein in cisplatin treatment-mediated cell cycle regulation was studied by Wang *et al.* Cisplatin is an antineoplastic drug used in treatment of many types of cancers where it binds to DNA to form intra- and inter-strand cross-links which will drive the cell towards apoptosis. However, presence of cancer cells resistant to cisplatin has undermined its curative potentials. XPC defect in the cisplatin treatment affected mostly cell cycle and cell proliferation-related genes as revealed by results of microarrays obtained from human normal fibroblasts and two individual XPC-defective cell lines. In fact, XPC defect also reduced p53 responses to cisplatin treatment and attenuated caspase-3 activation, p21 response and phosphorylated p53 expression. The decrease in p53 phosphorylation response might be that when XPC binds to DNA damage, it interacts with TFIIH where it forms a complex with it (Léveillard *et al.*, 1996). This enhanced interaction of TFIIH with p53 causes p53 phosphorylation by CDK7 (Ko *et al.*, 1997; Lu *et al.*, 1997) leading to subsequent activation of the p53 signal transduction pathway. Thus, XPC play a critical role in initiating the cisplatin DNA damaging treatment-mediated signal transduction process, resulting in activation of the p53 pathway and cell cycle arrest that allows DNA repair and apoptosis to take place. Therefore, These results reveal an important role of XPC in cancer prevention since XPC defect causes a failure in DNA damage recognition resulting in the damaged cells escaping from the cell cycle arrest and an increase in the risk of mutation accumulation and cancer incidence. Furthermore, up-regulation of XPC and DDB2 mRNA in p53 WT, but not p53 mutant glioma cells, in response to nimustine, a chloroethylating nitrosoureas agent, , indicates that p53 regulates a pathway that involves these DNA repair proteins (Batista *et al.*, 2007), in p53-dependent manner. For instance, upon UV-C irradiation in glioma cells, p53 WT cells show 9-fold increase in XPC protein level after 24h, while p53 mutant cells show only 1.1-fold increase. In p53-mutated glioma cells, the observed apoptosis upon UV-exposure is due to decreased efficiency in NER, reduced level of photoproduct repair, resulting in the persistence of DNA lesions which leads to activation of intrinsic apoptotic pathway, with Bcl-2 degradation and sustained Bax and Bak up-regulation. It has been also shown a decreased expression of XPC in human skin

fibroblasts after exposure to inorganic arsenite, which has been associated with many types of cancer such as bladder, kidney, lung and skin cancer. Arsenite exposure also leads to increased p53 expression that is the transcriptional activator of XPC and XPE. In addition, arsenite biomethylated metabolite monomethylarsonous acid (MMAIII) exerts more drastic effects that lead to diminished assembly of NER machinery. Both arsenite and MMAIII exposure decreases XPC and XPE gene expression, decreases XPC protein levels, increases p53 protein levels, and decreases XPC nuclear protein level and XPC association to local damage sites (Nollen et al., 2009). Moreover, XPC modulates the activity of p53 at the transcriptional level by inhibiting its degradation through the formation of hHR23B-p53 complex (Wu et al., 2010). Also, upon IR exposure, p53 played a major role in induction of XPC transcriptional levels and in the formation of an alternative shorter XPC transcript (Forrester et al., 2012). Furthermore, Barckhausen *et al.* showed that high chemoresistance witnessed in malignant melanomas is mediated by p53 and executed by XPC and DDB2. Indeed, administration of fotemustine (FM), a representative of alkylating DNA interstrand cross-linking (ICL) agents, during melanoma therapy does not induce cell death due to the counteraction by p53 that upregulates XPC and DDB2 genes. In fact, this robust and sustained response lasts for one week upon single drug administration, which in consequence will stimulate the repair of DNA ICLs. On the other hand, cells mutated for p53 are unable to repair ICLs, leading to prolonged ATM, ATR and CHK1 activation, and finally apoptosis. It's important to note that XPC and DDB2 induction also occurred upon treatment with other cross-linking anticancer drugs, such as cisplatin and mafosfamide, which confirms a general response of cancer cells to this group of chemotherapeutics. In summary, p53-dependent upregulation of XPC and DDB2 is a key mechanism upon genotoxic stress, whereby melanoma cells acquire resistance towards DNA cross-linking agents. However, it is worth noting that the increase of DDB2 and XPC does not impact the cellular response to methylating anticancer drugs such as dacarbazine (DTIC) and temozolomide (Barckhausen et al., 2014). Finally, a recent study by Krzeszinski et al. revealed a critical role for XPC in p53 regulation targeting it towards degradation through its interaction with Ub ligase MDM2. In the normal case in p53 degradation, MDM2 E3 recognizes and tags p53 with Ub chains. XPC binding to MDM2 leads to recruitment of the ubiquitylated p53 to the stable Rad23-XPC complex, where Rad23 is known as an ubiquitin receptor that delivers ubiquitylated p53 to the proteasome, thereby Rad23 interacts with the proteasome to degrade ubiquitylated p53. p53 is known to become stabilized following UV irradiation but can be rendered unstable by XPC overexpression, showing a critical role of XPC in p53 regulation (Krzeszinski et al.,

2014). Therefore, a negative feedback loop exists between XPC and p53 and a novel proteolytic role for XPC, other than DNA repair, has been discovered (**Figure 2**).

Further to this, Wang et al. demonstrated that XPC, independent of its known function in DNA repair, has a novel function as a potent enhancer of apoptosis in the absence of any influence by p53, in a p53 null background. However, this effect can be obscured by regular p53-mediated apoptosis. Indeed, XPC downregulates antiapoptotic casp-2S, short isoform of caspase-2, at both the RNA and protein levels through inhibition of its promoter activity and thus promotes DNA-damage induced activation of casp-9 and casp-6 which enhance cell death. Moreover, XPC overexpression in p53-deficient cancer cells resistant to cisplatin enhanced their sensitivity to cisplatin-induced apoptosis. These findings offer a mechanistic foundation to overcome the resistance of highly prevalent p53-deficient tumors to cell death induced by DNA-damaging therapeutic agents, by targeting strategies that inhibit the expression or function of casp-2S (Wang et al., 2012b).

3.2.XPC in cellular outcome

The prominent interaction of XPC with the tumor suppressor p53 affects the whole cellular outcome indirectly through p53 downstream signaling (**Figure 3**). Upon activation of p53, cells can undergo transient cell-cycle arrest as a result of induction of cyclin-dependant kinase inhibitor *p21*. In addition, cells can undergo apoptosis as a result of induction of pro-apoptotic *bcl2* gene family members that include *bax*, *puma* and *noxa*. Moreover, cells can undergo senescence through induction of the cyclin-dependant kinase inhibitor *p16^{Ink4a}* and the tumor suppressor gene *p19^{ARF}* (d' Adda di Fagagna, 2008; Sancar et al., 2004).

Several molecular and functional studies showed that XPC participates in the decision of cell-fate. A study by Rezvani and his team demonstrated that after five days of XPC-silencing in human keratinocytes, a peak of apoptosis was witnessed accompanied by an increase in expression of cell cycle inhibitors p16, p21 and p-Cdc2 (Rezvani et al., 2011b).

Other studies showed the interaction between XPC and tumor suppressor genes which reflects the importance of XPC in cellular maintenance, prevention of tumor growth, and determination of cellular outcome. For instance, XPC is a target for tumor suppressor ARF, which is derived from an alternative reading frame of the INK4A locus that encodes two tumor-suppressor proteins, *p16^{INK4a}* and *p19^{ARF}*. It has been proven that ARF mediates its gatekeeper tumor suppression activity by inhibiting murine double minute 2 (MDM2), which functions as a negative regulator of p53, which leads to activation of the p53 transcriptional

program resulting in cell-cycle arrest or apoptosis. On the other hand, ARF also possesses a p53-independent function in NER through its implication in the regulation of the E2F family, where DRTF polypeptide 1 (DP1) is critical for cell cycle progression. Through its ability to bind directly to DP1, ARF disrupts the interaction between E2F transcription factor 4 (E2F4) and DP1, which is the functional partner of E2F family of factors. As a result, the interaction of E2F4-p130 repressor complex with the promoter of XPC is reduced, which leads to higher levels of XPC expression (Dominguez-Brauer et al., 2009, 2010). Moreover, another study has shown that XPC is regulated by sirtuin 1 (SIRT1) which is a NAD-dependent longevity promoting deacetylase. In Caucasian skin, SIRT1 acts as tumor suppressor through its role in GG-NER where it increases XPC expression through inhibiting AKT dependent nuclear localization of E2F4-p130 repressor complex. Indeed, SIRT1 decreases the acetylation of PTEN, a negative regulator of PI3K/AKT activation, whereas the loss of SIRT1 increases AKT phosphorylation. PI3k/AKT pathway is a key oncogenic pathway that promotes cell growth and survival. It is also possible that SIRT1 deacetylates p130 and thus play important role in regulating the function of the E2F4-p130 repressor complex in XPC transcription (Ming et al., 2010). Therefore, upregulation of XPC by several tumor suppressor genes determines its participation in cell-fate decision.

Furthermore, XPC has been shown to affect telomere function, which itself has contrasting roles: inducing replicative senescence or promoting tumorigenesis. These roles may vary between cell types depending on the expression of telomerase enzyme, the level of mutations induced, and deficiency of related DNA repair pathways. A recent study showed that XPC is implicated in telomere stability (Stout and Blasco, 2013), a crucial requirement for the cell's well-being and survival. Indeed, when the telomere is shortened below a critical length, cells will undergo senescence or death (Ayouaz et al., 2008). Stout and Blasco used the two models XPC^{-/-} mutant mice and XPC^{-/-}G1-G3Terc^{-/-} double-mutant mice, which they exposed to UV radiation. Under chronic UV exposure, XPC^{-/-} skin displayed shorter telomeres proving that XPC is implicated in telomere stability. However, a double deficiency in XPC and in the telomerase (XPC^{-/-}G1-G3Terc^{-/-} model) showed aberrantly long telomeres, hence an activation of the alternative lengthening of telomeres (ALT). In addition, the double deficiency model displayed an elevated susceptibility for UV-induced p53 patches known to represent precursor lesions of carcinomas. Thus XPC has a role in telomere stability and seems to interact with telomerase where double absence of XPC and telomerase activates ALT pathway in a still unknown mechanism, leading to increased tumor incidence (Stout and Blasco, 2013).

4. XPC role in transcription

Apart from DNA repair, a new aspect for XPC function in the cell is transcription. May *et al.* showed that upon gene activation, RNA polymerase II transcription machinery assembles sequentially with the NER factors at the promoter. Indeed, in order to achieve optimal DNA demethylation, histone posttranslational modifications (PTMs) and efficient RNA synthesis, the assembly of NER factors to the transcription site is dependent on XPC protein, occurs in absence of exogenous genotoxic attack, and is sensitive to transcription inhibitors.. It is known that the formation of the transcription preinitiation complex (PIC), is accompanied by an important chromatin-remodeling phase including histones PTMs and active DNA demethylation of the promoter region that produce an euchromatin environment necessary to begin the synthesis of the primary transcript (Cedar and Bergman, 2009; Li et al., 2007). Upon formation of PIC, XPC is recruited and allows the sequential arrival of other NER factors in the following order CSB, XPA/RPA, XPG/XPF/Gadd45 α that associate with the transcription machinery leading to chromatin remodeling events. The absence of CSB (required for TCR) is not required for the recruitment of the other NER factors and mRNA synthesis. Although these factors may not be essential for PIC formation, they clearly fine-tune the transactivation to an optimal level. This optimization may be very important during development and can potentially explain the clinical symptoms observed in XP patients that cannot be explained by a DNA-repair deficiency (Le May et al., 2010b). It is essential to note that upon UV-induced DNA damage in Hela cells, recruitment of XPC and expression of non-UV inducible genes are dramatically delayed suggesting that a redistribution mechanism may redirect XPC from its transcription duty at promoter targets to the NER pathway(Le May et al., 2010b). Moreover, XPC has been reported to interact with chromatin remodeling factors. For example, XPC interacts with hSNF5, a component of the SWI/SNF ATP-dependent chromatin remodeling complex, in response to UV radiation (Ray et al., 2009). Furthermore, XPC potentially interacts weakly with p150, a subunit of chromatin assembly factor 1 (CAF-1) (Zhu et al., 2009).

Another evidence for XPC implication in transcription is that XPC acts as a multi-subunit stem cell coactivator complex (SCC), required for synergistic activation of Nanog gene by Oct4 and Sox2 (Fong *et al.*2011). Indeed, XPC interacts directly with Oct4 and Sox2 and is recruited to *Nanog* and *Oct4* promoters as well as the majority of genomic regions that are occupied by Oct4 and Sox2. Moreover depletion of XPC by knockdown strategy compromised both pluripotency in ESCs and somatic cell reprogramming of fibroblasts to iPS. Thus, XPC is required for ESC self renewal and efficient somatic cell reprogramming.

Therefore, XPC plays a role as a transcriptional coactivator in maintaining ESC pluripotency, in addition to its function of safeguarding the genome integrity (Fong et al., 2011). On the other hand, by using an inducible knockout strategy of C-terminal region of XPC in mouse ES cells, Ito et al. declared that the C-terminal region of XPC is dispensable for maintenance of pluripotency for Oct3/4 transcriptional activity but essential for DNA repair activity. The inducible deletion of XPC had no impact on self renewal of ES cells where no change in number of colonies was detected. In fact, the removal of the C-terminal region of XPC, including the interaction sites with Rad23 and Cctn2, showed weak impact on the gene expression profile of ES cells. Indeed, the functional *XPC-ΔC* ES cell lines retained proper gene expression profile as well as pluripotency, and where the proliferation was not affected. On the other hand, XPC knockout caused hypersensitivity to DNA damage. Nevertheless, the difference between the outcomes of the two studies has been attributed to the knockout/knockdown strategies used. The expression of the target gene is rapidly eliminated by the knockdown strategy, whereas by the knockout strategy, adaptation to the loss of the specific gene function might happen.

5. Conclusions

XPC has a widely known role in NER pathway, as a sensor of helical distortions, where it interacts with several proteins at the molecular level. However, XPC cross-talk with DNA repair pathways, other than NER, such as BER, HR, NHEJ, and MMR. In addition, XPC is a major player in DDR of the cell through its strong reciprocal interactions with tumor suppressor gene p53, and several other downstream molecules, that allow specification of cellular outcome such as apoptosis, senescence, or cell-cycle arrest. Outside of DNA damage, XPC participates in transcriptional processes in the cell where it is indispensable for the maintenance of the cell in general being a genomic caretaker and a cell fate decision maker. In the future, it would be of fundamental importance to consider XPC modifications in cancer patients before chemotherapy which can improve their chances of successful treatment and enhanced survival.

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Figure and Table legends

Figure 1. Molecular interplay of XPC during DNA damage recognition of NER. In GG-NER, XPC serves as damage sensor along with DDB2. Upon UV-exposure, XPC is subjected to phosphorylation by ATM and ATR kinases which activate its role in NER. WIP1 deactivates XPC, after repair of DNA damage, by catalyzing its dephosphorylation. XPC is ubiquitinated by DDB2-Cul4A-Rbx1 complex which activates its function. At the end of NER, ubiquitinated XPC can be directly degraded by interacting with 26S proteasome or recycled by OTUD4 which deubiquitinates it. XPC sumoylation protects XPC. RFN111 recognizes sumoylated XPC and target its ubiquitinylation. At the end of its function in NER, p97 segregase complex mediate the spatiotemporal regulation of XPC.

Figure 2. XPC molecular reciprocal interaction with p53. p53 regulates the transcription of XPC gene constitutively or more elaborately upon UV exposure. In its turn, XPC protein activates p53 by mediating its interaction with CDK7 kinase subunit of TFIIH complex which will phosphorylate p53 (right). On the other hand, XPC protein downregulates p53 protein by localizing p53 in close proximity to mdm2 protein which will ubiquitinylate p53 thereby allowing its recognition by HR23B that will target ubiquitinylated p53 towards degradation by 26S proteasome (left).

Figure 3. XPC determines cell fate through its implications in all stages of DDR response. This image represents molecules that have been reported to interact with XPC in the text. The DNA damage sensor XPC interacts with other DNA damage sensor proteins such as Ku70/80, H2AX, PARP and DDB2. Downstream DDR, XPC interacts with transducers such as ATM and ATR kinases inducing their activation by phosphorylation and also there is evidence suggesting an interaction between XPC and DNA-PK. Activated ATM and ATR will subsequently activate their substrates Chk2 and Chk1 respectively which will activate mediator BRCA1. BRCA1 activation will provoke DNA repair. On the other side, XPC also interacts with p53 which is the most important player in cellular outcome either activating it or mediating its degradation. XPC interacts with effector E2F4 where E2F4 inhibits XPC transcription. XPC downregulates effector pCdc2 leading to inhibition of cell cycle progression. Downstream, XPC interacts with p21 directly or indirectly through p53 mediating cell-cycle arrest. Similarly, XPC interaction with p16 and p19 provokes cell senescence. XPC also mediate apoptosis through its interaction with antiapoptotic BCL2 and

apoptotic BAX proteins. In addition, XPC affect cellular outcome by influencing cellular transcription process. Therefore, defect in XPC could perturb the whole cellular integrity and target cells toward death or towards carcinogenesis. Note: The red marking of some of the grey items signal an existence of feedback interaction with XPC.

Table 1. XPC crosstalk with diverse DNA repair pathways. Upon induction of various types of DNA damage, XPC mediate complex molecular interactions with NER, BER, HR, NHEJ and MMR pathways. A list of molecules of each DNA repair pathway is presented that also interacts with XPC.

Figure 1:

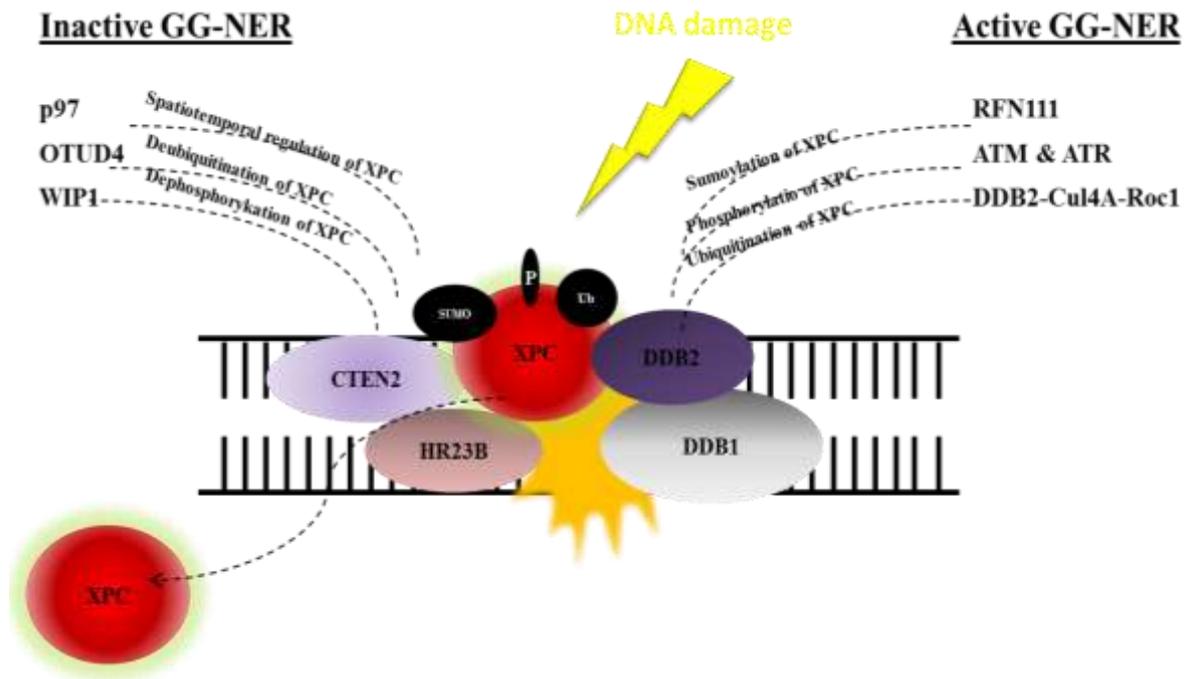


Figure 2:

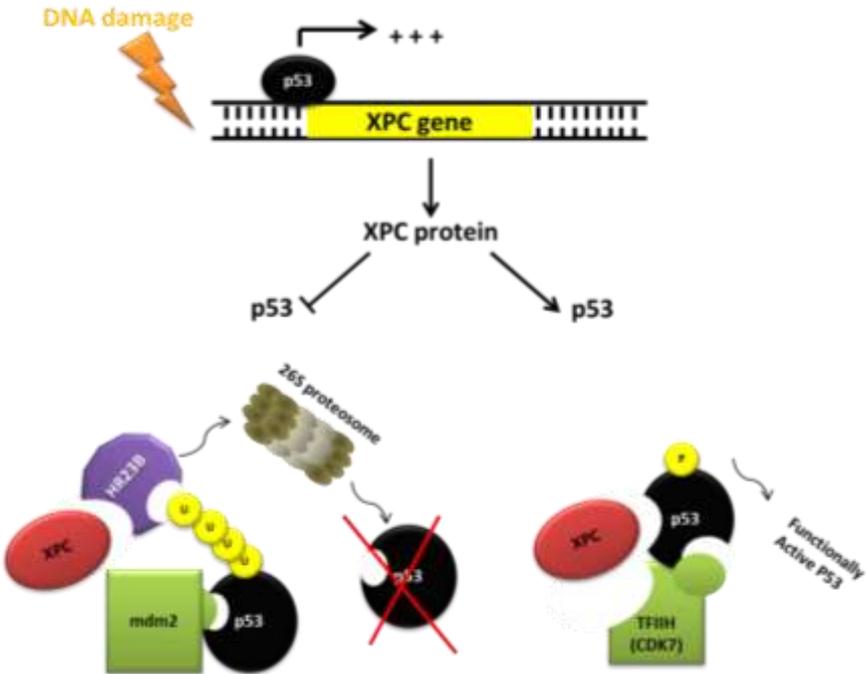


Figure 3:

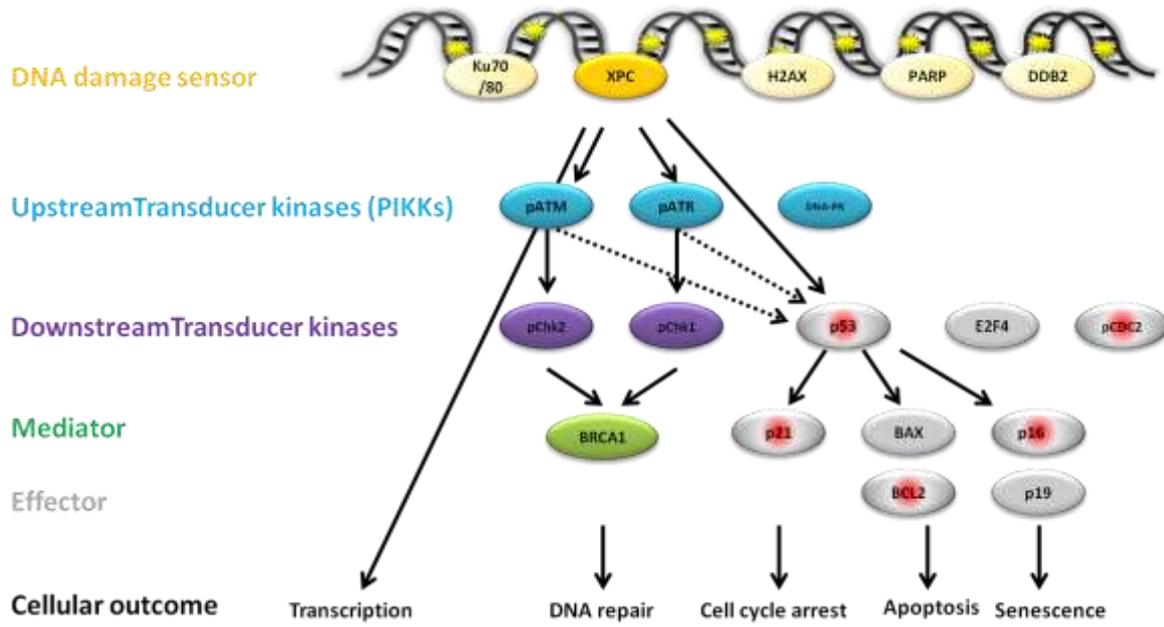


Table 1:

DNA damage repair pathway	Interacting proteins	Function	Effect of its interaction with XPC
NER	HR23B	DNA damage recognition	HR23B inhibit XPC polyubiquitinylation & degradation by 26S proteasome
	Cetrin 2	DNA damage recognition	CETR2 confers stability to XPC
	TFIIH	DNA unwinding	XPC recruits TFIIH to unwind DNA
	DDB2	DNA damage recognition	Both form complex to scan DNA for distortions
	RPA	Correct positioning of the repair proteins around the lesion	XPC participates in stability of RPA
	XPA	Verification of DNA lesion	XPC participates in stability of XPA
	P97	Ubiquitin-dependent protein quality control	Spatiotemporal regulation of XPC by p97
BER	TAG	Release 3-methyladenine	XPC stimulate its activity
	TDG	Release G/T mismatches	XPC stimulate its activity
	OGG1	Release 8-hydroxyguanosine products	XPC stimulate its activity
	SMUG1	Releases uracil from SSBs and DSBs	XPC stimulate its activity
	APE	Codes for apurinic/ apyrimidinic (AP) endonuclease function	Combination of XPC mutation with Apex heterozygosity increases the predisposition to skin cancer by decreasing its latency time
	PARP1	Inhibition of PARP1 delays repair of SSBs by BER	XPC deficiency cause slight increase in PARP-1 and PARP inhibition disrupts interaction of XPC with DDB2 and decreased its stability at lesion site
	MMR	MSh2	Part of MutS activity responsible for mismatch recognition of base-base mismatches, small and large IDLs
MutSβ		Part of MutS activity responsible for mismatch recognition of large IDLs	MutSβ and XPC bind in a complex to psoralen ICL under high protein concentration.
MLh1		Part of MutL responsible for downstreaming MMR events by coupling to MutS complex	XPC deficient cells show increase in mLh1 transcription
NHEJ	PKcs	Tethering the ends of the DSBs	XPC deficient cells show increase in RNA transcript and protein level
	Ku70/XRCC6	Binding to the exposed DNA termini of the DSB	XPC deficient cells show increase in RNA transcript and protein level
	XRCC4	Associates with DNA ligase IV to mediate ligation of DNA ends	XPC deficient cells show increase in XRCC4 protein level and its association occurs at lower doses under stress
	Ligase IV	Mediates ligation of DNA ends	XPC deficient cells show increase in protein level and its association occurs at lower doses under stress
HR	ATR	Mediate H2AX phosphorylation	XPC recruits ATR to DNA damage site
	ATM	DNA damage sensor	XPC activates ATM by physical interaction enabling its association with genomic DNA
	γH2AX	Focus formation at sites of double-strand breaks	XPC influence γH2AX levels and XPC deficient cells show much lower levels of H2AX phosphorylation
	Rad51	Executes DNA sequence homology search, mediates DNA strand invasion reaction	XPC deficient cells showed a marked recruitment of Rad51 to the damage site

References

- D'Adda di Fagagna, F. (2008). Living on a break: cellular senescence as a DNA-damage response. *Nat. Rev. Cancer* 8, 512–522.
- Adimoolam, S., and Ford, J.M. (2002). p53 and DNA damage-inducible expression of the xeroderma pigmentosum group C gene. *Proc. Natl. Acad. Sci. U. S. A.* 99, 12985–12990.
- Araki, M., Masutani, C., Takemura, M., Uchida, A., Sugasawa, K., Kondoh, J., Ohkuma, Y., and Hanaoka, F. (2001). Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair. *J. Biol. Chem.* 276, 18665–18672.
- Arlett, C.F., Plowman, P.N., Rogers, P.B., Parris, C.N., Abbaszadeh, F., Green, M.H.L., McMillan, T.J., Bush, C., Foray, N., and Lehmann, A.R. (2006). Clinical and cellular ionizing radiation sensitivity in a patient with xeroderma pigmentosum. *Br. J. Radiol.* 79, 510–517.
- Auclair, Y., Rouget, R., Affar, E.B., and Drobetsky, E.A. (2008). ATR kinase is required for global genomic nucleotide excision repair exclusively during S phase in human cells. *Proc. Natl. Acad. Sci. U. S. A.* 105, 17896–17901.
- Auclair, Y., Rouget, R., and Drobetsky, E.A. (2009). ATR kinase as master regulator of nucleotide excision repair during S phase of the cell cycle. *Cell Cycle Georget. Tex* 8, 1865–1871.
- Ayouaz, A., Raynaud, C., Heride, C., Revaud, D., and Sabatier, L. (2008). Telomeres: Hallmarks of radiosensitivity. *Biochimie* 90, 60–72.
- Barckhausen, C., Roos, W.P., Naumann, S.C., and Kaina, B. (2014). Malignant melanoma cells acquire resistance to DNA interstrand cross-linking chemotherapeutics by p53-triggered upregulation of DDB2/XPC-mediated DNA repair. *Oncogene* 33, 1964–1974.
- Barnes, L., Dumas, M., Juan, M., Noblesse, E., Tesniere, A., Schnebert, S., Guillot, B., and Molès, J.-P. (2010). γ H2AX, an Accurate Marker That Analyzes UV Genotoxic Effects on Human Keratinocytes and on Human Skin. *Photochem. Photobiol.* 86, 933–941.
- Bartek, J., and Lukas, J. (2007). DNA damage checkpoints: from initiation to recovery or adaptation. *Curr. Opin. Cell Biol.* 19, 238–245.
- Batista, L.F.Z., Roos, W.P., Christmann, M., Menck, C.F.M., and Kaina, B. (2007). Differential sensitivity of malignant glioma cells to methylating and chloroethylating anticancer drugs: p53 determines the switch by regulating XPC, ddb2, and DNA double-strand breaks. *Cancer Res.* 67, 11886–11895.
- Bernardes de Jesus, B.M., Bjørås, M., Coin, F., and Egly, J.M. (2008). Dissection of the molecular defects caused by pathogenic mutations in the DNA repair factor XPC. *Mol. Cell. Biol.* 28, 7225–7235.
- Brooks, C.L., and Gu, W. (2011). p53 regulation by ubiquitin. *FEBS Lett.* 585, 2803–2809.
- Burma, S., Chen, B.P., Murphy, M., Kurimasa, A., and Chen, D.J. (2001). ATM Phosphorylates Histone H2AX in Response to DNA Double-strand Breaks. *J. Biol. Chem.* 276, 42462–42467.

Cadet, J., Douki, T., Gasparutto, D., and Ravanat, J.-L. (2003). Oxidative damage to DNA: formation, measurement and biochemical features. *Mutat. Res. Mol. Mech. Mutagen.* 531, 5–23.

Cedar, H., and Bergman, Y. (2009). Linking DNA methylation and histone modification: patterns and paradigms. *Nat. Rev. Genet.* 10, 295–304.

Chen, Z., Yang, J., Wang, G., Song, B., Li, J., and Xu, Z. (2007). Attenuated expression of xeroderma pigmentosum group C is associated with critical events in human bladder cancer carcinogenesis and progression. *Cancer Res.* 67, 4578–4585.

Colton, S.L., Xu, X.S., Wang, Y.A., and Wang, G. (2006). The involvement of ataxia-telangiectasia mutated protein activation in nucleotide excision repair-facilitated cell survival with cisplatin treatment. *J. Biol. Chem.* 281, 27117–27125.

Dantuma, N.P., Heinen, C., and Hoogstraten, D. (2009). The ubiquitin receptor Rad23: at the crossroads of nucleotide excision repair and proteasomal degradation. *DNA Repair* 8, 449–460.

D’Errico, M., Parlanti, E., Teson, M., de Jesus, B.M.B., Degan, P., Calcagnile, A., Jaruga, P., Bjørås, M., Crescenzi, M., Pedrini, A.M., et al. (2006). New functions of XPC in the protection of human skin cells from oxidative damage. *EMBO J.* 25, 4305–4315.

Despras, E., Pfeiffer, P., Salles, B., Calsou, P., Kuhfittig-Kulle, S., Angulo, J.F., and Biard, D.S.F. (2007). Long-term XPC Silencing Reduces DNA Double-Strand Break Repair. *Cancer Res.* 67, 2526–2534.

Dominguez-Brauer, C., Chen, Y.-J., Brauer, P.M., Pimkina, J., and Raychaudhuri, P. (2009). ARF stimulates XPC to trigger nucleotide excision repair by regulating the repressor complex of E2F4. *EMBO Rep.* 10, 1036–1042.

Dominguez-Brauer, C., Brauer, P.M., Chen, Y.-J., Pimkina, J., and Raychaudhuri, P. (2010). Tumor suppression by ARF: gatekeeper and caretaker. *Cell Cycle Georget. Tex* 9, 86–89.

Fong, Y.W., Inouye, C., Yamaguchi, T., Cattoglio, C., Grubisic, I., and Tjian, R. (2011). A DNA repair complex functions as an Oct4/Sox2 coactivator in embryonic stem cells. *Cell* 147, 120–131.

Ford, J.M., and Hanawalt, P.C. (1997). Expression of wild-type p53 is required for efficient global genomic nucleotide excision repair in UV-irradiated human fibroblasts. *J. Biol. Chem.* 272, 28073–28080.

Forrester, H.B., Li, J., Hovan, D., Ivashkevich, A.N., and Sprung, C.N. (2012). DNA repair genes: alternative transcription and gene expression at the exon level in response to the DNA damaging agent, ionizing radiation. *PloS One* 7, e53358.

Fréchet, M., Warrick, E., Vioux, C., Chevallier, O., Spatz, A., Benhamou, S., Sarasin, A., Bernerd, F., and Magnaldo, T. (2008). Overexpression of matrix metalloproteinase 1 in dermal fibroblasts from DNA repair-deficient/cancer-prone xeroderma pigmentosum group C patients. *Oncogene* 27, 5223–5232.

Friedberg, E.C., Aguilera, A., Gellert, M., Hanawalt, P.C., Hays, J.B., Lehmann, A.R., Lindahl, T., Lowndes, N., Sarasin, A., and Wood, R.D. (2006). DNA repair: from molecular mechanism to human disease. *DNA Repair* 5, 986–996.

Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J., Saijo, M., Drapkin, R., Kisselev, A.F., Tanaka, K., and Nakatani, Y. (2003). The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. *Cell* 113, 357–367.

Hanasoge, S., and Ljungman, M. (2007). H2AX phosphorylation after UV irradiation is triggered by DNA repair intermediates and is mediated by the ATR kinase. *Carcinogenesis* 28, 2298–2304.

Hoogervorst, E.M., van Oostrom, C.T.M., Beems, R.B., van Benthem, J., van den Berg, J., van Kreijl, C.F., Vos, J.G., de Vries, A., and van Steeg, H. (2005). 2-AAF-induced tumor development in nucleotide excision repair-deficient mice is associated with a defect in global genome repair but not with transcription coupled repair. *DNA Repair* 4, 3–9.

Hoogstraten, D., Bergink, S., Ng, J.M.Y., Verbiest, V.H.M., Luijsterburg, M.S., Geverts, B., Raams, A., Dinant, C., Hoeijmakers, J.H.J., Vermeulen, W., et al. (2008). Versatile DNA damage detection by the global genome nucleotide excision repair protein XPC. *J. Cell Sci.* 121, 2850–2859.

Kadekaro, A.L., Chen, J., Yang, J., Chen, S., Jameson, J., Swope, V.B., Cheng, T., Kadakia, M., and Abdel-Malek, Z. (2012). Alpha-Melanocyte-Stimulating Hormone Suppresses Oxidative Stress through a p53-Mediated Signaling Pathway in Human Melanocytes. *Mol. Cancer Res.* 10, 778–786.

Kassam, S.N., and Rainbow, A.J. (2007). Deficient base excision repair of oxidative DNA damage induced by methylene blue plus visible light in xeroderma pigmentosum group C fibroblasts. *Biochem. Biophys. Res. Commun.* 359, 1004–1009.

Kim, J.K., Patel, D., and Choi, B.S. (1995). Contrasting structural impacts induced by cis-syn cyclobutane dimer and (6-4) adduct in DNA duplex decamers: implication in mutagenesis and repair activity. *Photochem. Photobiol.* 62, 44–50.

Kinner, A., Wu, W., Staudt, C., and Iliakis, G. (2008). Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. *Nucleic Acids Res.* 36, 5678–5694.

Ko, L.J., Shieh, S.Y., Chen, X., Jayaraman, L., Tamai, K., Taya, Y., Prives, C., and Pan, Z.Q. (1997). p53 is phosphorylated by CDK7-cyclin H in a p36MAT1-dependent manner. *Mol. Cell. Biol.* 17, 7220–7229.

Kraemer, K.H., DiGiovanna, J.J., and Peck, G.L. (1992). Chemoprevention of skin cancer in xeroderma pigmentosum. *J. Dermatol.* 19, 715–718.

Krzyszinski, J.Y., Choe, V., Shao, J., Bao, X., Cheng, H., Luo, S., Huo, K., and Rao, H. (2014). XPC promotes MDM2-mediated degradation of the p53 tumor suppressor. *Mol. Biol. Cell* 25, 213–221.

Lee, Y.-C., Cai, Y., Mu, H., Broyde, S., Amin, S., Chen, X., Min, J.-H., and Geacintov, N.E. The relationships between XPC binding to conformationally diverse DNA adducts and their excision by the human NER system: Is there a correlation? *DNA Repair*.

Léveillard, T., Andera, L., Bissonnette, N., Schaeffer, L., Bracco, L., Egly, J.M., and Wasyluk, B. (1996). Functional interactions between p53 and the TFIIH complex are affected by tumour-associated mutations. *EMBO J.* 15, 1615–1624.

- Li, B., Carey, M., and Workman, J.L. (2007). The role of chromatin during transcription. *Cell* 128, 707–719.
- Liu, S.-Y., Wen, C.-Y., Lee, Y.-J., and Lee, T.-C. (2010). XPC silencing sensitizes glioma cells to arsenic trioxide via increased oxidative damage. *Toxicol. Sci. Off. J. Soc. Toxicol.* 116, 183–193.
- Lombard, D.B., Chua, K.F., Mostoslavsky, R., Franco, S., Gostissa, M., and Alt, F.W. (2005). DNA repair, genome stability, and aging. *Cell* 120, 497–512.
- Lu, H., Fisher, R.P., Bailey, P., and Levine, A.J. (1997). The CDK7-cycH-p36 complex of transcription factor IIIH phosphorylates p53, enhancing its sequence-specific DNA binding activity in vitro. *Mol. Cell. Biol.* 17, 5923–5934.
- Lubin, A., Zhang, L., Chen, H., White, V.M., and Gong, F. (2014). A human XPC protein interactome--a resource. *Int. J. Mol. Sci.* 15, 141–158.
- Mah, L.-J., El-Osta, A., and Karagiannis, T.C. (2010). γ H2AX: a sensitive molecular marker of DNA damage and repair. *Leukemia* 24, 679–686.
- Matsumoto, M., Yaginuma, K., Igarashi, A., Imura, M., Hasegawa, M., Iwabuchi, K., Date, T., Mori, T., Ishizaki, K., Yamashita, K., et al. (2007). Perturbed gap-filling synthesis in nucleotide excision repair causes histone H2AX phosphorylation in human quiescent cells. *J. Cell Sci.* 120, 1104–1112.
- Matsuoka, S., Ballif, B.A., Smogorzewska, A., McDonald, E.R., 3rd, Hurov, K.E., Luo, J., Bakalarski, C.E., Zhao, Z., Solimini, N., Lerenthal, Y., et al. (2007). ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316, 1160–1166.
- Le May, N., Mota-Fernandes, D., Vélez-Cruz, R., Iltis, I., Biard, D., and Egly, J.M. (2010). NER Factors Are Recruited to Active Promoters and Facilitate Chromatin Modification for Transcription in the Absence of Exogenous Genotoxic Attack. *Mol. Cell* 38, 54–66.
- Meira, L.B., Reis, A.M., Cheo, D.L., Nahari, D., Burns, D.K., and Friedberg, E.C. (2001). Cancer predisposition in mutant mice defective in multiple genetic pathways: uncovering important genetic interactions. *Mutat. Res.* 477, 51–58.
- Melis, J.P.M., Wijnhoven, S.W.P., Beems, R.B., Roodbergen, M., van den Berg, J., Moon, H., Friedberg, E., van der Horst, G.T.J., Hoeijmakers, J.H.J., Vijg, J., et al. (2008). Mouse Models for Xeroderma Pigmentosum Group A and Group C Show Divergent Cancer Phenotypes. *Cancer Res.* 68, 1347–1353.
- Menoni, H., Hoeijmakers, J.H.J., and Vermeulen, W. (2012). Nucleotide excision repair-initiating proteins bind to oxidative DNA lesions in vivo. *J. Cell Biol.* 199, 1037–1046.
- Miao, F., Bouziane, M., Dammann, R., Masutani, C., Hanaoka, F., Pfeifer, G., and O'Connor, T.R. (2000). 3-Methyladenine-DNA glycosylase (MPG protein) interacts with human RAD23 proteins. *J. Biol. Chem.* 275, 28433–28438.
- Min, J.-H., and Pavletich, N.P. (2007). Recognition of DNA damage by the Rad4 nucleotide excision repair protein. *Nature* 449, 570–575.

- Ming, M., Shea, C.R., Guo, X., Li, X., Soltani, K., Han, W., and He, Y.-Y. (2010). Regulation of global genome nucleotide excision repair by SIRT1 through xeroderma pigmentosum C. *Proc. Natl. Acad. Sci. U. S. A.* *107*, 22623–22628.
- Molinier, J., Ramos, C., Fritsch, O., and Hohn, B. (2004). CENTRIN2 Modulates Homologous Recombination and Nucleotide Excision Repair in Arabidopsis. *Plant Cell Online* *16*, 1633–1643.
- Mullaart, E., Lohman, P.H.M., Berends, F., and Vijg, J. (1990). DNA damage metabolism and aging. *Mutat. Res.* *237*, 189–210.
- Nahari, D., McDaniel, L.D., Task, L.B., Daniel, R.L., Velasco-Miguel, S., and Friedberg, E.C. (2004). Mutations in the Trp53 gene of UV-irradiated XPC mutant mice suggest a novel XPC-dependent DNA repair process. *DNA Repair* *3*, 379–386.
- Nguyen, T.-A., Slattery, S.D., Moon, S.-H., Darlington, Y.F., Lu, X., and Donehower, L.A. (2010). The oncogenic phosphatase WIP1 negatively regulates nucleotide excision repair. *DNA Repair* *9*, 813–823.
- Nishi, R., Okuda, Y., Watanabe, E., Mori, T., Iwai, S., Masutani, C., Sugasawa, K., and Hanaoka, F. (2005). Centrin 2 stimulates nucleotide excision repair by interacting with xeroderma pigmentosum group C protein. *Mol. Cell. Biol.* *25*, 5664–5674.
- Nollen, M., Ebert, F., Moser, J., Mullenders, L.H.F., Hartwig, A., and Schwerdtle, T. (2009). Impact of arsenic on nucleotide excision repair: XPC function, protein level, and gene expression. *Mol. Nutr. Food Res.* *53*, 572–582.
- Nouspikel, T. (2011). Multiple roles of ubiquitination in the control of nucleotide excision repair. *Mech. Ageing Dev.* *132*, 355–365.
- Park, H., Zhang, K., Ren, Y., Nadji, S., Sinha, N., Taylor, J.-S., and Kang, C. (2002). Crystal structure of a DNA decamer containing a cis-syn thymine dimer. *Proc. Natl. Acad. Sci. U. S. A.* *99*, 15965–15970.
- Poulsen, S.L., Hansen, R.K., Wagner, S.A., van Cuijk, L., van Belle, G.J., Streicher, W., Wikström, M., Choudhary, C., Houtsmuller, A.B., Marteijn, J.A., et al. (2013). RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. *J. Cell Biol.* *201*, 797–807.
- Puumalainen, M.-R., Lessel, D., Rütthemann, P., Kaczmarek, N., Bachmann, K., Ramadan, K., and Naegeli, H. (2014). Chromatin retention of DNA damage sensors DDB2 and XPC through loss of p97 segregase causes genotoxicity. *Nat. Commun.* *5*.
- Ray, A., Mir, S.N., Wani, G., Zhao, Q., Battu, A., Zhu, Q., Wang, Q.-E., and Wani, A.A. (2009). Human SNF5/INI1, a component of the human SWI/SNF chromatin remodeling complex, promotes nucleotide excision repair by influencing ATM recruitment and downstream H2AX phosphorylation. *Mol. Cell. Biol.* *29*, 6206–6219.
- Ray, A., Milum, K., Battu, A., Wani, G., and Wani, A.A. (2013). NER initiation factors, DDB2 and XPC, regulate UV radiation response by recruiting ATR and ATM kinases to DNA damage sites. *DNA Repair* *12*, 273–283.

Reis, A.M., Cheo, D.L., Meira, L.B., Greenblatt, M.S., Bond, J.P., Nahari, D., and Friedberg, E.C. (2000). Genotype-specific Trp53 mutational analysis in ultraviolet B radiation-induced skin cancers in XPC and XPC Trp53 mutant mice. *Cancer Res.* *60*, 1571–1579.

Rezvani, H.R., Rossignol, R., Ali, N., Benard, G., Tang, X., Yang, H.S., Jouary, T., de Verneuil, H., Taïeb, A., Kim, A.L., et al. (2011a). XPC silencing in normal human keratinocytes triggers metabolic alterations through NOX-1 activation-mediated reactive oxygen species. *Biochim. Biophys. Acta* *1807*, 609–619.

Rezvani, H.R., Kim, A.L., Rossignol, R., Ali, N., Daly, M., Mahfouf, W., Bellance, N., Taïeb, A., de Verneuil, H., Mazurier, F., et al. (2011b). XPC silencing in normal human keratinocytes triggers metabolic alterations that drive the formation of squamous cell carcinomas. *J. Clin. Invest.* *121*, 195–211.

Robu, M., Shah, R.G., Petitclerc, N., Brind'Amour, J., Kandan-Kulangara, F., and Shah, G.M. (2013). Role of poly(ADP-ribose) polymerase-1 in the removal of UV-induced DNA lesions by nucleotide excision repair. *Proc. Natl. Acad. Sci. U. S. A.* *110*, 1658–1663.

Sancar, A. (1996). DNA excision repair. *Annu. Rev. Biochem.* *65*, 43–81.

Sancar, A., Lindsey-Boltz, L.A., Unsal-Kaçmaz, K., and Linn, S. (2004). Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu. Rev. Biochem.* *73*, 39–85.

Shimizu, Y., Iwai, S., Hanaoka, F., and Sugawara, K. (2003). Xeroderma pigmentosum group C protein interacts physically and functionally with thymine DNA glycosylase. *EMBO J.* *22*, 164–173.

Singh, S.K., Szulik, M.W., Ganguly, M., Khutsishvili, I., Stone, M.P., Marky, L.A., and Gold, B. (2011). Characterization of DNA with an 8-oxoguanine modification. *Nucleic Acids Res.* *39*, 6789–6801.

Smith, J., Mun Tho, L., Xu, N., and Gillespie, D. (2010). Chapter 3 - The ATM–Chk2 and ATR–Chk1 Pathways in DNA Damage Signaling and Cancer. In *Advances in Cancer Research*, George F. Vande Woude and George Klein, ed. (Academic Press), pp. 73–112.

Stout, G.J., and Blasco, M.A. (2013). Telomere length and telomerase activity impact the UV sensitivity syndrome xeroderma pigmentosum C. *Cancer Res.* *73*, 1844–1854.

Strom, C.E., Johansson, F., Uhlen, M., Szigyarto, C.A.-K., Erixon, K., and Helleday, T. (2011). Poly (ADP-ribose) polymerase (PARP) is not involved in base excision repair but PARP inhibition traps a single-strand intermediate. *Nucleic Acids Res.* *39*, 3166–3175.

Sugawara, K. (2006). UV-induced ubiquitylation of XPC complex, the UV-DDB-ubiquitin ligase complex, and DNA repair. *J. Mol. Histol.* *37*, 189–202.

Sugawara, K., Okamoto, T., Shimizu, Y., Masutani, C., Iwai, S., and Hanaoka, F. (2001). A multistep damage recognition mechanism for global genomic nucleotide excision repair. *Genes Dev.* *15*, 507–521.

Sugawara, K., Shimizu, Y., Iwai, S., and Hanaoka, F. (2002). A molecular mechanism for DNA damage recognition by the xeroderma pigmentosum group C protein complex. *DNA Repair* *1*, 95–107.

Sugasawa, K., Okuda, Y., Saijo, M., Nishi, R., Matsuda, N., Chu, G., Mori, T., Iwai, S., Tanaka, K., Tanaka, K., et al. (2005). UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. *Cell* 121, 387–400.

Sugasawa, K., Akagi, J., Nishi, R., Iwai, S., and Hanaoka, F. (2009). Two-Step Recognition of DNA Damage for Mammalian Nucleotide Excision Repair: Directional Binding of the XPC Complex and DNA Strand Scanning. *Mol. Cell* 36, 642–653.

Swope, V., Alexander, C., Starner, R., Schwemberger, S., Babcock, G., and Abdel-Malek, Z.A. (2014). Significance of the melanocortin 1 receptor in the DNA damage response of human melanocytes to ultraviolet radiation. *Pigment Cell Melanoma Res.*

Takedachi, A., Saijo, M., and Tanaka, K. (2010). DDB2 complex-mediated ubiquitylation around DNA damage is oppositely regulated by XPC and Ku and contributes to the recruitment of XPA. *Mol. Cell. Biol.* 30, 2708–2723.

Tanaka, T., Halicka, H.D., Traganos, F., and Darzynkiewicz, Z. (2006). Phosphorylation of Histone H2AX on Ser 139 and Activation of ATM During Oxidative Burst in Phorbol Ester-Treated Human Leukocytes. *Cell Cycle* 5, 2671–2675.

Tang, J.Y., Hwang, B.J., Ford, J.M., Hanawalt, P.C., and Chu, G. (2000). Xeroderma pigmentosum p48 gene enhances global genomic repair and suppresses UV-induced mutagenesis. *Mol. Cell* 5, 737–744.

Wade, M., Wang, Y.V., and Wahl, G.M. (2010). The p53 orchestra: Mdm2 and Mdmx set the tone. *Trends Cell Biol.* 20, 299–309.

Wang, G., Chuang, L., Zhang, X., Colton, S., Dombkowski, A., Reiners, J., Diakiw, A., and Xu, X.S. (2004). The initiative role of XPC protein in cisplatin DNA damaging treatment-mediated cell cycle regulation. *Nucleic Acids Res.* 32, 2231–2240.

Wang, Q.-E., Zhu, Q., Wani, G., El-Mahdy, M.A., Li, J., and Wani, A.A. (2005). DNA repair factor XPC is modified by SUMO-1 and ubiquitin following UV irradiation. *Nucleic Acids Res.* 33, 4023–4034.

Wang, Q.-E., Praetorius-Ibba, M., Zhu, Q., El-Mahdy, M.A., Wani, G., Zhao, Q., Qin, S., Patnaik, S., and Wani, A.A. (2007). Ubiquitylation-independent degradation of Xeroderma pigmentosum group C protein is required for efficient nucleotide excision repair. *Nucleic Acids Res.* 35, 5338–5350.

Wang, Q.-E., Han, C., Zhang, B., Sabapathy, K., and Wani, A.A. (2012). Nucleotide excision repair factor XPC enhances DNA damage-induced apoptosis by downregulating the antiapoptotic short isoform of caspase-2. *Cancer Res.* 72, 666–675.

Ward, I.M., Minn, K., and Chen, J. (2004). UV-induced Ataxia-telangiectasia-mutated and Rad3-related (ATR) Activation Requires Replication Stress. *J. Biol. Chem.* 279, 9677–9680.

Wijnhoven, S.W., Kool, H.J., Mullenders, L.H., van Zeeland, A.A., Friedberg, E.C., van der Horst, G.T., van Steeg, H., and Vrieling, H. (2000). Age-dependent spontaneous mutagenesis in XPC mice defective in nucleotide excision repair. *Oncogene* 19, 5034–5037.

Wood, R.D. (1997). Nucleotide excision repair in mammalian cells. *J. Biol. Chem.* 272, 23465–23468.

Wu, X., Shell, S.M., Yang, Z., and Zou, Y. (2006). Phosphorylation of Nucleotide Excision Repair Factor Xeroderma Pigmentosum Group A by Ataxia Telangiectasia Mutated and Rad3-Related-Dependent Checkpoint Pathway Promotes Cell Survival in Response to UV Irradiation. *Cancer Res.* 66, 2997–3005.

Wu, Y.-H., Wu, T.-C., Liao, J.-W., Yeh, K.-T., Chen, C.-Y., and Lee, H. (2010). p53 dysfunction by xeroderma pigmentosum group C defects enhance lung adenocarcinoma metastasis via increased MMP1 expression. *Cancer Res.* 70, 10422–10432.

Yajima, H., Lee, K.-J., Zhang, S., Kobayashi, J., and Chen, B.P.C. (2009). DNA double-strand break formation upon UV-induced replication stress activates ATM and DNA-PKcs kinases. *J. Mol. Biol.* 385, 800–810.

Zhao, J., Jain, A., Iyer, R.R., Modrich, P.L., and Vasquez, K.M. (2009). Mismatch repair and nucleotide excision repair proteins cooperate in the recognition of DNA interstrand crosslinks. *Nucleic Acids Res.* 37, 4420–4429.

Zhu, Q., Wani, G., Arab, H.H., El-Mahdy, M.A., Ray, A., and Wani, A.A. (2009). Chromatin restoration following nucleotide excision repair involves the incorporation of ubiquitinated H2A at damaged genomic sites. *DNA Repair* 8, 262–273.

ANNEX 3

Review article in preparation

XPC cop at the crossroads of tumorigenesis

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Short title: **XPC a genomic caretaker**

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Abstract

Xeroderma pigmentosum group C, XPC, has been widely known as a DNA damage recognition factor of bulky adducts and as an initiator of GG-NER pathway. XPC defect has been profoundly correlated with wide spectrum of tumors. Indeed, skin cancer susceptibility upon XPC defect, internal tumor and leukemia susceptibility suggest tremendous functions of XPC outside of its ancient known role in NER. Finally, XPC can be considered at the same time as a genomic caretaker.

1. Introduction

The cell represents a crowded factory of complex, tightly regulated, coordinated and overlapping molecular pathways that are governed by a DNA double helix that harbors the whole genetic material. The integrity of DNA double helix is fundamental for the survival of cells, tissues, organs and the organism. Throughout life, DNA integrity is continuously threatened by external insults such as ultraviolet (UV) radiation, ionizing radiations (IR), exposure to radioactive substances, chemical reagents, and chemotherapeutic drugs. These factors can induce base modifications, inter- and intra-strand crosslinks (ICL), single and double-strand breaks (SSBs, DSBs), which leads to genomic instability (Mullaart et al., 1990). Cells can also be threatened by internal insults created by the ongoing metabolic processes inside the cell that continuously generate abundant endogenous lesions such as depurination, depyrimidination and deamination of the DNA components (Cadet et al., 2003; Friedberg et al., 2006). Both endogenous and exogenous lesions can result in persistent DNA damage that will perturb cellular processes and eventually lead to mutations and disturbed homeostasis which may result in cancer and age-related diseases. Therefore, living cells have developed several ways to fix this DNA damage through repair pathways. Indeed, defects in one of the genes involved in DNA repair mechanisms can result in severe syndromes due to the loss of genomic stability.

Xeroderma pigmentosum (XP) is a rare disorder, transmitted in an autosomal recessive manner, generated by deficiency in a DNA repair pathway called nucleotide excision repair (NER). In fact, XP was the first human NER-deficient disease to be discovered and refers to parchment pigmented skin (Cleaver et al., 2009). XP disease is characterized by an early onset of cutaneous abnormalities at the age of 1.5 years due to a cellular hypersensitivity to UV radiation resulting from a defect in DNA repair (English and Swerdlow, 1987). Related symptoms include photosensitivity, cutaneous atrophy and telangiectasia, actinic keratosis, malignant skin neoplasms, in addition to ocular and neurological abnormalities. XP disease is an example of an expanding family of human NER-diseases. Indeed, there are seven complementation groups that have been identified to be involved in XP disease. These include *XP-A* through *XP-G* that are caused by mutations in genes that code for XPA to XPG proteins plus a variant form *XPV* that is caused by mutation in a gene that codes for polymerase- η (Cleaver, 2005b).

XP-C or also called Xeroderma pigmentosum group C is one of the most common complementation groups of XP. It represents a key weapon used by the cell to defend itself

against DNA damage. XPC was first identified in 1933 and characterized in 1991 (Bootsma and Hoeijmakers, 1991). It is the most common form of XP in the white population, accounting for over a third of all cases (Li et al., 1993). This complementation group corresponds to mutations in the gene coding for the XPC protein that is localized on the short arm of chromosome 3, band 25.1. Mutations within the *XPC* gene are by far the most common genetic alteration found in European and North African XP patients. XPC protein, constituting of 940 amino acids, is majorly known as an essential DNA damage recognition protein of the GG-NER pathway where it harbors domains that can bind to damaged DNA and repair factors.

2. XPC conventional role in NER

The NER mechanism is one of the most essential repair pathways since it repairs a broad spectrum of bulky DNA lesions and helix-distorting damages induced by several damaging agents. The most relevant substrates of NER are cyclobutane pyrimidine dimers (CPDs) and pyrimidine-(6,4)-pyrimidone products (6-4PP) produced by UV light, a widely known physical DNA damaging agent (Lo et al., 2005). Both CPD and 6-4PP lesions in the DNA are characterized by the formation of covalent bonds between adjacent pyrimidines on the same DNA strand, and differ only by the number and position of these bonds (Friedberg et al., 1995). In addition, exposure to numerous chemicals or alkylating agents generate helix-distorting bulky adducts such as polycyclic aromatic hydrocarbons (Boer and Hoeijmakers, 2000) which are repaired by NER. Indeed, in order to eliminate the helix-distorting damage the NER process employs a battery of over 40 proteins acting in successive steps (Neumann et al., 2005). NER is divided into two sub pathways, global genome NER (GG-NER) and transcription-coupled NER (TC-NER) (Hanawalt, 2002). These 2 sub pathways initiate in a divergent manner during the first step of damage recognition, thereafter, they both proceed along the same processes. GG-NER recognizes and removes lesions throughout the entire genome rendering it a relatively slow process, while TC-NER removes the lesions in the transcribed strand of active genes, a fast efficient process. GG-NER and TC-NER differ in their mode of DNA damage recognition. TC-NER is initiated only when transcription is blocked by stalled RNA polymerase II, which is relieved by the assembly of a protein complex comprising two proteins: Cockayne Syndrome complementation group A (CSA) and B (CSB). CSB protein is a member of the SWI/SNF family of DNA-dependent ATPases and is implicated in chromatin remodeling whereas CSA is a cofactor for SCF-type ubiquitin ligase. CSB is responsible for the recruitment of the successive components of the NER machinery to the damaged site while CSA is required to recruit the chromatin remodeling

factors (Nakatsu et al., 2000). On the other hand, GG-NER is initiated when XPC recognizes and binds to damage in non-transcribed regions of the genome as a heterotrimeric complex XPC–HR23B–centrin 2 along with XPE as a damage-specific DNA binding protein 2 (DDB2)–DDB1 heterodimer (Dip et al., 2004). Then XPC recruits the pre-incision complex (Araújo et al., 2001).

Nevertheless, the role of XPC in DNA lesion recognition has been shown recently to include a dark side (Puumalainen et al., 2014). In fact, the beneficial DNA repair response triggered by XPC and DDB2 depends critically on a dynamic spatio-temporal regulation of their homeostasis mediated by ubiquitin-dependent p97 segregase complex. Recruitment of p97 to UV lesions is primarily dependent on DDB2 and to a lesser degree on XPC. Failure to remove ubiquitylated XPC and DDB2 due to p97 deficiency leads to impaired DNA excision repair and provokes formation of chromosomal aberrations. Thus, XPC functions as a double-edged sword since it is essential to trigger a beneficial DNA repair activity but may become detrimental to the genome, if allowed to accumulate in damaged chromatin without control by the p97 segregase (Puumalainen et al., 2014).

Following DNA damage recognition in TC-NER and GG-NER, the two sub pathways overlap proceeding along the same axis. Indeed, the second step in the NER process is DNA unwinding, which is mediated by TFIIH complex containing XPB and XPD helicases that unwind the DNA helix, facilitating the entrance of the pre-incision complex to the lesion site (Goosen, 2010). Verification of DNA lesion is mediated by subsequent binding of XPA (Hermanson-Miller and Turchi, 2002) that presents docking sites for XPG and XPF-ERCC1 endonucleases and binding of RPA, a single-strand DNA binding complex, that facilitates the correct positioning of the repair proteins around the lesion (Krasikova et al., 2010; Park and Choi, 2006). Dual DNA incisions are made by XPG and ERCC1-XPF, at 3' and 5' ends respectively, (Staresinic et al., 2009) resulting in the excision of a single strand fragment of 24-32 nucleotides containing the damaged site (Hess et al., 1997). After excision of damaged fragment, the gap is filled by DNA polymerases (Pol δ , Pol ϵ , and Pol κ) whose functions are facilitated by proliferating cell nuclear antigen (PCNA), RPA, and replication factor C. The final step involves DNA ligation by DNA ligase that closes the 3' nick leading to restoration of the original DNA fragment. Therefore, XPC is the initiator of GG-NER by its DNA damage recognition capacity.

3. XPC generates divergent tumor spectrum

3.1. Skin cancer

The strong implication of XPC in carcinogenesis has been the focus of research since a long time. It is widely known that XPC defect leads to skin cancer due to its conventional role as a sensor of helical distortions in GG-NER pathway of NER. For instance, XPC defect in humans gives rise to XP disease (Neumann et al., 2005; Wood et al., 2001) with hypersensitivity to UV light, skin atrophy, actinic keratoses and increased risk of skin cancer by 1000-fold (Kraemer et al., 1987). The $XPC^{-/-}$ mouse model, which is only defective for GG-NER and not for TC-NER, showed highly predisposition to UV-induced skin cancer, similar to humans (Berg et al., 1998; Cheo et al., 1996, 2000; Friedberg et al., 1999; Sands et al., 1995). It also showed higher spontaneous and radiation-induced mutations rates in comparison to wild-type ($XPC^{+/+}$) and heterozygous ($XPC^{+/-}$) mice (Miccoli et al., 2007). Moreover, $XPC^{+/-}$ mice showed higher susceptibility to UV-induced skin cancer compared to wild type littermates, which was not the case in $Xpa^{+/-}$ mice (Friedberg et al., 2000). XPC defect also leads to spontaneous formation of squamous cell carcinoma when immunodeficient mice were injected with XPC-silenced keratinocytes (Rezvani et al., 2011b).

3.2. Internal tumors

Profound *in vitro* evidence, using XPC-silenced cells, and *in vivo*, using XPC knockout models, show that XPC defect is implicated in development of internal tumors. Nevertheless, in humans the incidence of internal tumors due to XPC defect remains limited and less prevalent than skin tumors. This is mainly due to the fact that internal tissues are more protected than skin to exposure of damaging agents such as UV and chemicals and also that XPC patients are short-lived whereas internal tumors require long time to be formed.

In humans, the first evidence of XPC implication in internal tumors is that XP patients show an increased risk of developing internal cancer by 10- to 20-fold (Kraemer et al., 1987). It is thought that XPC patients develop skin cancers prior to age 20 and develop internal cancers with aging. Moreover, three known single nucleotide polymorphisms (SNPs) of XPC (Lys939Gln, Ala499Val and poly (AT) insertion/deletion polymorphism (PAT^{+/-})) have been targets for extensive pharmacogenomic studies that aimed to explore association of XPC mutations with cancer susceptibility in humans. Several meta-analysis studies showed that Lys939Gln SNP is significantly associated with increased risk of lung cancer (He et al., 2013; Jin et al., 2014b, 2014b), bladder cancer (Dai et al., 2014; He et al., 2013; Zhang et al., 2013), digestive system cancer (Jiang et al., 2012), thyroid cancer (Santos et al., 2013), and

colorectal cancer (He et al., 2013). In addition, meta-analysis studies on Ala499Val SNP showed that it's significantly associated with breast cancer (Dai et al., 2014; He et al., 2013; McCullough et al., 2014; Wang et al., 2014), bladder cancer (He et al., 2013) and head and neck cancer (Zhang et al., 2014). Moreover, studies on PAT polymorphism revealed significant association with susceptibility to urinary system cancer (Dai et al., 2013), bladder cancer (Dai et al., 2014), and head and neck cancer (Zhang et al., 2014). Furthermore, some XPC patients don't only show symptoms related to skin abnormalities, common for XP disease, but also exhibit neurological abnormalities (Khan et al., 2009). For instance, an unusual XPC patient was reported to have neurologic symptoms and features of systemic lupus erythematosus (Hananian and Cleaver, 1980). Also, a 4-year-old boy of Korean ancestry with XPC possessed unusual neurologic features including hyperactivity and autistic features in addition to sun sensitivity and multiple cutaneous neoplasms (Khan et al., 1998). Two young XPC patients were reported to have primary internal tumors (Giglia et al., 1998) and Hollander et al. reported potential allelic loss of XPC in many of the human lung tumors (Hollander et al., 2005a). Finally, one study showed myelosuppression in an XPC patient (Salob et al., 1992).

Furthermore, a strong functional correlation exists between XPC deficiency and bladder carcinogenesis. Indeed, human tissues derived from bladder cancer patients showed strong correlation between bladder cancer progression and attenuated XPC protein expression (Chen et al., 2007). There was also a strong correlation between XPC deficiency, p53 mutation, and the degree of malignancy of bladder tumors. In addition, bladder cancer cells with low-levels of XPC protein exhibited a decreased DNA repair capability and were resistant to cisplatin treatment (Chen et al., 2007). Another study showed that XPC defect was associated with bladder cancer higher pathological grade and metastasis (Yang et al., 2010). Interestingly, bladder cancer patients with defect in XPC had shorter survival rates than patients without XPC defect. In addition, XPC protein levels were reduced due to epigenetic hypermethylation of *XPC* gene (Yang et al., 2010). Moreover, it was shown that reduced levels of XPC protein in bladder cancer patients results from gene silencing by histone deacetylases (HDACs) (Xu et al., 2011). Finally, tumor progression and metastasis were also observed in lung cancer patients with XPC defects (Wu et al., 2010).

In mice, XPC deficiency showed a wide spectrum of internal tumors. Indeed, *XPC*^{-/-} mice possess a high predisposition to the induction of lung and liver cancers upon treatment with 2-acetylaminofluorene (2-AAF) and NAOH-2-AAF (Meira et al., 2001a). In addition, *XPC*^{-/-} mice in a mixed genetic background showed an extremely high and significantly increased

lung tumor incidence where at the time of death, 100% of the $XPC^{-/-}$ mice harbored lung tumors although the survival was not affected (Hollander et al., 2005a). Consistently, a similar study on $XPC^{-/-}$ mice, in a congenic C57BL/6J background, revealed a significant increase in lung tumor and a trend toward increased liver tumors whereas $Xpa^{-/-}$ mice did not show an increase in lung tumor but had a significantly increased incidence of hepatocellular liver tumor (Melis et al., 2008a). Concomitantly, $Xpc^{-/-}$ mice showed a strong exclusive increase in mutational load in the lungs during aging in comparison to their wild type controls and to $Xpa^{-/-}$ mice. Furthermore, upon exposure to the oxidative stressors diethylhexyl phthalate (DEHP) and paraquat for 39 weeks, $Xpc^{-/-}$ mice had a significant increase in liver lipofuscin, an in vivo marker for oxidative stress levels, and a two-fold increase in mutational load in the liver, in comparison to wild type and Xpa mice. On the other hand, exposure for 12 weeks did not result in any significant changes showing that mutations arise in a slowly cumulative fashion. The effect of XPC deficiency on liver mutagenesis was associated with absence of anti-oxidant glutathione response and an upregulation in cell cycle progression in XPC mice (Melis et al., 2011, 2013b). Therefore, XPC deficiency contributes to slow accumulation of mutations upon oxidative DNA damage whereas appearance of internal tumors during XPC deficiency occurs later in age.

3.3. Hematopoietic diseases

In humans, several pharmacogenomics studies have shown that XPC polymorphisms is associated with increased risk of leukemias and altered susceptibility to genotoxic effects of treatment. Indeed, XPC Ala499Val reduced overall and disease-free survival among 170 adult de-novo AML patients with intermediate cytogenetics, treated with induction chemotherapy. In fact, the risk of dying among patients for Ala499Val XPC, carrying one or two variant alleles (CT/TT), was associated with 78% increased mortality, compared to the wild-type genotype (CC) (HR 1.78, $p = 0.02$) (Strom et al., 2010). Furthermore, Ala499Val and Lys939Gln XPC SNPs were associated to imatinib treatment response in 92 early chronic phase CML patients. Wild-type haplotypes of both SNs were associated with a better response to imatinib than heterozygous carriers (Guillem et al., 2010).

In mice, several studies provided proofs for XPC's role in diseases of the hematopoietic system. It was shown that spontaneous mutant frequencies at the *Hprt* gene in T lymphocytes of the spleen increased significantly in $Xpc^{-/-}$ mice and that this increase was enhanced with aging compared to $Xpa^{-/-}$ and $Csb^{-/-}$ mice. Aged $XPC^{+/-}$ mice also exhibited a significantly elevated mutant frequency reflecting a haplo-insufficiency of the *XPC* locus (Wijnhoven et

al., 2000). Also, *Xpc*^{-/-} mice show a significant increase in acidophilic macrophage pneumonia, which is not apparent in *Xpa*^{-/-} mice (Melis et al., 2008a). In addition, long term paraquat exposure lead to a significant increase in lymphoid hyperplasia and edema incidence in lungs of *Xpc*^{-/-} mice (Melis et al., 2013b). Moreover, BM of *Xpc*^{-/-} mice exhibited 10-fold greater sensitivity to carboplatin that, similar to cisplatin, produces mainly 1,2 and 1,3 platinum di-adducts, compared to BMs of wild-type. Furthermore, upon carboplatin treatment, XPC deficient mice had a decreased survival compared to wild-type mice. This effect of XPC on BM cell survival was contributed to its impact on cell cycle since XPC deficient BM cells were defective in G1 checkpoints but had an increased accumulation in G2, in comparison to wild type. Moreover, colony-forming ability of XPC deficient BM cells was decreased 3-fold, compared to wild type, even in the absence of carboplatin (Fischer et al., 2009). It was also shown an increased XPC response *in vitro* and *in vivo* at the transcriptional level in LSK HSCs extracted from male 129/SvJ mice under exposure to 1,4-benzoquinone (1,4-BQ). This compound was shown to cause DNA lesions that lead to changes in HSCs, progressive decline of hematopoietic function, generation of leukemic clones and onset of various disorders including aplastic anemia, myelodysplastic syndrome and leukemia (Faiola et al., 2004). Recently, Beerman et al. demonstrated that XPC is downregulated in quiescent HSCs which leads to an increased DNA damage with aging, however, XPC is upregulated as HSCs enter into cell cycle leading to repair of strand breaks (Beerman et al., 2014).

Therefore, the wide spectrum of tumors generated by XPC deficiency cannot all be attributed to malfunctioned GG-NER, which strongly suggests that XPC has important non-NER activity. Consistent with this assumption, a novel study allowed to elucidate the interactome of XPC, using a high-throughput Yeast Two Hybrid screening (Lubin et al., 2014). Indeed, 12% of the discovered protein interactome had roles in DNA repair and replication, 14% had roles in proteolysis and post-translational modifications, 8% had roles in transcription regulation, 20% had roles in signal transduction and the majority 32% had roles in metabolism. In consistence, the Lys939Gln allele of XPC, which has been shown to associate with high risk of cancers, has in fact a wild-type NER activity (Khan et al., 2000).

4. Conclusions

XPC has a widely known role in NER pathway, as a sensor of helical distortions, where it interacts with several proteins at the molecular level. Several lines of evidence reveal that XPC is linked to tumorigenesis which is not limited to skin cancer but also to progression of internal tumors and even leukemia. In the future, it would be of fundamental importance to

consider XPC modifications in cancer patients before chemotherapy which can improve their chances of successful treatment and enhanced survival.

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References

- Araújo, S.J., Nigg, E.A., and Wood, R.D. (2001). Strong functional interactions of TFIIH with XPC and XPG in human DNA nucleotide excision repair, without a preassembled repairosome. *Mol. Cell. Biol.* *21*, 2281–2291.
- Beerman, I., Seita, J., Inlay, M.A., Weissman, I.L., and Rossi, D.J. (2014). Quiescent Hematopoietic Stem Cells Accumulate DNA Damage during Aging that Is Repaired upon Entry into Cell Cycle. *Cell Stem Cell*.
- Berg, R.J., Ruven, H.J., Sands, A.T., de Gruijl, F.R., and Mullenders, L.H. (1998). Defective global genome repair in XPC mice is associated with skin cancer susceptibility but not with sensitivity to UVB induced erythema and edema. *J. Invest. Dermatol.* *110*, 405–409.
- Boer, J. de, and Hoeijmakers, J.H.J. (2000). Nucleotide excision repair and human syndromes. *Carcinogenesis* *21*, 453–460.
- Bootsma, D., and Hoeijmakers, J.H. (1991). The genetic basis of xeroderma pigmentosum. *Ann. Génétique* *34*, 143–150.
- Cadet, J., Douki, T., Gasparutto, D., and Ravanat, J.-L. (2003). Oxidative damage to DNA: formation, measurement and biochemical features. *Mutat. Res. Mol. Mech. Mutagen.* *531*, 5–23.
- Chen, Z., Yang, J., Wang, G., Song, B., Li, J., and Xu, Z. (2007). Attenuated expression of xeroderma pigmentosum group C is associated with critical events in human bladder cancer carcinogenesis and progression. *Cancer Res.* *67*, 4578–4585.
- Cheo, D.L., Meira, L.B., Hammer, R.E., Burns, D.K., Doughty, A.T., and Friedberg, E.C. (1996). Synergistic interactions between XPC and p53 mutations in double-mutant mice: neural tube abnormalities and accelerated UV radiation-induced skin cancer. *Curr. Biol. CB* *6*, 1691–1694.
- Cheo, D.L., Meira, L.B., Burns, D.K., Reis, A.M., Issac, T., and Friedberg, E.C. (2000). Ultraviolet B radiation-induced skin cancer in mice defective in the XPC, Trp53, and Apex (HAP1) genes: genotype-specific effects on cancer predisposition and pathology of tumors. *Cancer Res.* *60*, 1580–1584.
- Cleaver, J.E. (2005). Cancer in xeroderma pigmentosum and related disorders of DNA repair. *Nat. Rev. Cancer* *5*, 564–573.
- Cleaver, J.E., Lam, E.T., and Revet, I. (2009). Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. *Nat. Rev. Genet.* *10*, 756–768.
- Dai, Q.-S., Hua, R.-X., Zhang, R., Huang, Y.-S., Hua, Z.-M., Yun, C.T., Zeng, R.-F., and Long, J.-T. (2013). Poly (AT) deletion/insertion polymorphism of the XPC gene contributes to urinary system cancer susceptibility: a meta-analysis. *Gene* *528*, 335–342.
- Dai, Q.-S., Hua, R.-X., Zeng, R.-F., Long, J.-T., and Peng, Z.-W. (2014). XPC gene polymorphisms contribute to bladder cancer susceptibility: a meta-analysis. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* *35*, 447–453.
- Dip, R., Camenisch, U., and Naegeli, H. (2004). Mechanisms of DNA damage recognition and strand discrimination in human nucleotide excision repair. *DNA Repair* *3*, 1409–1423.

- English, J.S., and Swerdlow, A.J. (1987). The risk of malignant melanoma, internal malignancy and mortality in xeroderma pigmentosum patients. *Br. J. Dermatol.* *117*, 457–461.
- Faiola, B., Fuller, E.S., Wong, V.A., Pluta, L., Abernethy, D.J., Rose, J., and Recio, L. (2004). Exposure of hematopoietic stem cells to benzene or 1,4-benzoquinone induces gender-specific gene expression. *Stem Cells Dayt. Ohio* *22*, 750–758.
- Fischer, J.L., Kumar, M.A.S., Day, T.W., Hardy, T.M., Hamilton, S., Besch-Williford, C., Safa, A.R., Pollok, K.E., and Smith, M.L. (2009). The XPC gene markedly affects cell survival in mouse bone marrow. *Mutagenesis* *24*, 309–316.
- Friedberg, E.C., Walker, G.C., and Siede, W. (1995). *DNA Repair and Mutagenesis* (ASM Press).
- Friedberg, E.C., Cheo, D.L., Meira, L.B., and Reis, A.M. (1999). Cancer predisposition in mutant mice defective in the XPC DNA repair gene. *Prog. Exp. Tumor Res.* *35*, 37–52.
- Friedberg, E.C., Bond, J.P., Burns, D.K., Cheo, D.L., Greenblatt, M.S., Meira, L.B., Nahari, D., and Reis, A.M. (2000). Defective nucleotide excision repair in XPC mutant mice and its association with cancer predisposition. *Mutat. Res.* *459*, 99–108.
- Friedberg, E.C., Aguilera, A., Gellert, M., Hanawalt, P.C., Hays, J.B., Lehmann, A.R., Lindahl, T., Lowndes, N., Sarasin, A., and Wood, R.D. (2006). DNA repair: from molecular mechanism to human disease. *DNA Repair* *5*, 986–996.
- Giglia, G., Dumaz, N., Drougard, C., Avril, M.F., Daya-Grosjean, L., and Sarasin, A. (1998). p53 mutations in skin and internal tumors of xeroderma pigmentosum patients belonging to the complementation group C. *Cancer Res.* *58*, 4402–4409.
- Goosen, N. (2010). Scanning the DNA for damage by the nucleotide excision repair machinery. *DNA Repair* *9*, 593–596.
- Hananian, J., and Cleaver, J.E. (1980). Xeroderma pigmentosum exhibiting neurological disorders and systemic lupus erythematosus. *Clin. Genet.* *17*, 39–45.
- Hanawalt, P.C. (2002). Subpathways of nucleotide excision repair and their regulation. *Oncogene* *21*, 8949–8956.
- He, J., Shi, T.-Y., Zhu, M.-L., Wang, M.-Y., Li, Q.-X., and Wei, Q.-Y. (2013). Associations of Lys939Gln and Ala499Val polymorphisms of the XPC gene with cancer susceptibility: a meta-analysis. *Int. J. Cancer* *133*, 1765–1775.
- Hermanson-Miller, I.L., and Turchi, J.J. (2002). Strand-Specific Binding of RPA and XPA to Damaged Duplex DNA†. *Biochemistry (Mosc.)* *41*, 2402–2408.
- Hess, M.T., Schwitter, U., Petretta, M., Giese, B., and Naegeli, H. (1997). Bipartite substrate discrimination by human nucleotide excision repair. *Proc. Natl. Acad. Sci.* *94*, 6664–6669.
- Hollander, M.C., Philburn, R.T., Patterson, A.D., Velasco-Miguel, S., Friedberg, E.C., Linnoila, R.I., and Fornace, A.J., Jr (2005). Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. *Proc. Natl. Acad. Sci. U. S. A.* *102*, 13200–13205.

Jiang, X., Zhou, L.-T., Zhang, S.-C., and Chen, K. (2012). XPC Polymorphism Increases Risk of Digestive System Cancers: Current Evidence from A Meta-Analysis. *Chin. J. Cancer Res. Chung-Kuo Yen Cheng Yen Chiu* 24, 181–189.

Jin, B., Dong, Y., Zhang, X., Wang, H., and Han, B. (2014). Association of XPC Polymorphisms and Lung Cancer Risk: A Meta-Analysis. *PloS One* 9, e93937.

Khan, S.G., Levy, H.L., Legerski, R., Quackenbush, E., Reardon, J.T., Emmert, S., Sancar, A., Li, L., Schneider, T.D., Cleaver, J.E., et al. (1998). Xeroderma pigmentosum group C splice mutation associated with autism and hypoglycinemia. *J. Invest. Dermatol.* 111, 791–796.

Khan, S.G., Metter, E.J., Tarone, R.E., Bohr, V.A., Grossman, L., Hedayati, M., Bale, S.J., Emmert, S., and Kraemer, K.H. (2000). A new xeroderma pigmentosum group C poly(AT) insertion/deletion polymorphism. *Carcinogenesis* 21, 1821–1825.

Khan, S.G., Oh, K.-S., Emmert, S., Imoto, K., Tamura, D., Digiovanna, J.J., Shahlavi, T., Armstrong, N., Baker, C.C., Neuburg, M., et al. (2009). XPC initiation codon mutation in xeroderma pigmentosum patients with and without neurological symptoms. *DNA Repair* 8, 114–125.

Kraemer, K.H., Lee, M.M., and Scotto, J. (1987). Xeroderma pigmentosum. Cutaneous, ocular, and neurologic abnormalities in 830 published cases. *Arch. Dermatol.* 123, 241–250.

Krasikova, Y.S., Rechkunova, N.I., Maltseva, E.A., Petrusseva, I.O., and Lavrik, O.I. (2010). Localization of xeroderma pigmentosum group A protein and replication protein A on damaged DNA in nucleotide excision repair. *Nucleic Acids Res.* 38, 8083–8094.

Li, L., Bales, E.S., Peterson, C.A., and Legerski, R.J. (1993). Characterization of molecular defects in xeroderma pigmentosum group C. *Nat. Genet.* 5, 413–417.

Lo, H.-L., Nakajima, S., Ma, L., Walter, B., Yasui, A., Ethell, D.W., and Owen, L.B. (2005). Differential biologic effects of CPD and 6-4PP UV-induced DNA damage on the induction of apoptosis and cell-cycle arrest. *BMC Cancer* 5, 135.

Lubin, A., Zhang, L., Chen, H., White, V.M., and Gong, F. (2014). A human XPC protein interactome--a resource. *Int. J. Mol. Sci.* 15, 141–158.

McCullough, L.E., Santella, R.M., Cleveland, R.J., Millikan, R.C., Olshan, A.F., North, K.E., Bradshaw, P.T., Eng, S.M., Terry, M.B., Shen, J., et al. (2014). Polymorphisms in DNA repair genes, recreational physical activity and breast cancer risk. *Int. J. Cancer J. Int. Cancer* 134, 654–663.

Meira, L.B., Reis, A.M., Cheo, D.L., Nahari, D., Burns, D.K., and Friedberg, E.C. (2001). Cancer predisposition in mutant mice defective in multiple genetic pathways: uncovering important genetic interactions. *Mutat. Res.* 477, 51–58.

Melis, J.P.M., Wijnhoven, S.W.P., Beems, R.B., Roodbergen, M., van den Berg, J., Moon, H., Friedberg, E., van der Horst, G.T.J., Hoeijmakers, J.H.J., Vijg, J., et al. (2008). Mouse Models for Xeroderma Pigmentosum Group A and Group C Show Divergent Cancer Phenotypes. *Cancer Res.* 68, 1347–1353.

Melis, J.P.M., Luijten, M., Mullenders, L.H.F., and van Steeg, H. (2011). The role of XPC: implications in cancer and oxidative DNA damage. *Mutat. Res.* 728, 107–117.

Melis, J.P.M., Kuiper, R.V., Zwart, E., Robinson, J., Pennings, J.L.A., van Oostrom, C.T.M., Luijten, M., and van Steeg, H. (2013). Slow accumulation of mutations in XPC^{-/-} mice upon induction of oxidative stress. *DNA Repair* 12, 1081–1086.

Miccoli, L., Burr, K.L.-A., Hickenbotham, P., Friedberg, E.C., Angulo, J.F., and Dubrova, Y.E. (2007). The combined effects of xeroderma pigmentosum C deficiency and mutagens on mutation rates in the mouse germ line. *Cancer Res.* 67, 4695–4699.

Mullaart, E., Lohman, P.H.M., Berends, F., and Vijg, J. (1990). DNA damage metabolism and aging. *Mutat. Res.* 237, 189–210.

Nakatsu, Y., Asahina, H., Citterio, E., Rademarkers, S., Vermeulen, W., Kamiuchi, S., Yeo, J.-P., Khaw, M.-C., Saijo, M., Kodo, N., et al. (2000). XAB2, a novel tetratricopeptide repeat protein, involved in transcription-coupled DNA repair and transcription. *J. Biol. Chem.*

Neumann, A.S., Sturgis, E.M., and Wei, Q. (2005). Nucleotide excision repair as a marker for susceptibility to tobacco-related cancers: a review of molecular epidemiological studies. *Mol. Carcinog.* 42, 65–92.

Park, C.-J., and Choi, B.-S. (2006). The protein shuffle. Sequential interactions among components of the human nucleotide excision repair pathway. *FEBS J.* 273, 1600–1608.

Puumalainen, M.-R., Lessel, D., Rütthemann, P., Kaczmarek, N., Bachmann, K., Ramadan, K., and Naegeli, H. (2014). Chromatin retention of DNA damage sensors DDB2 and XPC through loss of p97 segregase causes genotoxicity. *Nat. Commun.* 5.

Rezvani, H.R., Kim, A.L., Rossignol, R., Ali, N., Daly, M., Mahfouf, W., Bellance, N., Taïeb, A., de Verneuil, H., Mazurier, F., et al. (2011). XPC silencing in normal human keratinocytes triggers metabolic alterations that drive the formation of squamous cell carcinomas. *J. Clin. Invest.* 121, 195–211.

Salob, S.P., Webb, D.K., and Atherton, D.J. (1992). A child with xeroderma pigmentosum and bone marrow failure. *Br. J. Dermatol.* 126, 372–374.

Sands, A.T., Abuin, A., Sanchez, A., Conti, C.J., and Bradley, A. (1995). High susceptibility to ultraviolet-induced carcinogenesis in mice lacking XPC. *Nature* 377, 162–165.

Santos, L.S., Gomes, B.C., Gouveia, R., Silva, S.N., Azevedo, A.P., Camacho, V., Manita, I., Gil, O.M., Ferreira, T.C., Limbert, E., et al. (2013). The role of CCNH Val270Ala (rs2230641) and other nucleotide excision repair polymorphisms in individual susceptibility to well-differentiated thyroid cancer. *Oncol. Rep.* 30, 2458–2466.

Staresincic, L., Fagbemi, A.F., Enzlin, J.H., Gourdin, A.M., Wijgers, N., Dunand-Sauthier, I., Giglia-Mari, G., Clarkson, S.G., Vermeulen, W., and Schärer, O.D. (2009). Coordination of dual incision and repair synthesis in human nucleotide excision repair. *EMBO J.* 28, 1111–1120.

Wang, Y., Li, Z., Liu, N., and Zhang, G. (2014). Association between CCND1 and XPC polymorphisms and bladder cancer risk: a meta-analysis based on 15 case-control studies. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* 35, 3155–3165.

Wijnhoven, S.W., Kool, H.J., Mullenders, L.H., van Zeeland, A.A., Friedberg, E.C., van der Horst, G.T., van Steeg, H., and Vrieling, H. (2000). Age-dependent spontaneous mutagenesis in XPC mice defective in nucleotide excision repair. *Oncogene* 19, 5034–5037.

Wood, R.D., Mitchell, M., Sgouros, J., and Lindahl, T. (2001). Human DNA repair genes. *Science* 291, 1284–1289.

Wu, Y.-H., Wu, T.-C., Liao, J.-W., Yeh, K.-T., Chen, C.-Y., and Lee, H. (2010). p53 dysfunction by xeroderma pigmentosum group C defects enhance lung adenocarcinoma metastasis via increased MMP1 expression. *Cancer Res.* 70, 10422–10432.

Xu, X.S., Wang, L., Abrams, J., and Wang, G. (2011). Histone deacetylases (HDACs) in XPC gene silencing and bladder cancer. *J. Hematol. Oncol.* 4, 17.

Yang, J., Xu, Z., Li, J., Zhang, R., Zhang, G., Ji, H., Song, B., and Chen, Z. (2010). XPC epigenetic silence coupled with p53 alteration has a significant impact on bladder cancer outcome. *J. Urol.* 184, 336–343.

Zhang, Y., Wang, X., Zhang, W., and Gong, S. (2013). An association between XPC Lys939Gln polymorphism and the risk of bladder cancer: a meta-analysis. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* 34, 973–982.

Zhang, Y., Li, Z., Zhong, Q., Zhou, W., Chen, X., Chen, X., Fang, J., and Huang, Z. (2014). Polymorphisms of the XPC gene may contribute to the risk of head and neck cancer: a meta-analysis. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* 35, 3917–3931.

