Cell Stem Cell
Review

DNA-Damage Response in Tissue-Specific and Cancer Stem Cells

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Recent studies have shown that tissue-specific stem cells (SCs) found throughout the body respond differentially to DNA damage. In this review, we will discuss how different SC populations sense and functionally respond to DNA damage, identify various common and distinct mechanisms utilized by tissue-specific SCs to address DNA damage, and describe how these mechanisms can impact SC genomic integrity by potentially promoting aging, tissue atrophy, and/or cancer development. Finally, we will discuss how similar mechanisms operate in cancer stem cells (CSCs) and can mediate resistance to chemo- and radiotherapy.

Stem cells (SCs) are often referred to as the mother of all cells, meaning they sit at the apex of a cellular hierarchy and, upon differentiation, give rise to all the mature cells of a tissue (Rossi et al., 2008). More specifically, SCs are described as having the unique capacity to self-renew, in order to establish and replenish the SC pool, and also to differentiate, thereby generating progeny that carry out specific tissue functions. SCs are essential for specification and morphogenesis of tissues during embryonic development (organogenesis) and for the maintenance and repair of adult tissues throughout life by replacing cells lost during normal tissue turnover (homeostasis) or after injury. Although tissue-specific SCs are found in many highly regenerative organs, such as blood, skin, and the digestive tract, they are also found in nonrenewing organs such as muscle, where they allow repair after tissue damage.

Like every other cell in the body, SCs must constantly contend with genotoxic insults arising from both endogenous chemical reactions, such as reactive oxygen species (ROS) generated by cellular metabolism, and exogenous insults coming from their surrounding environment (Sancar et al., 2004). It has been estimated that every cell undergoes about 100,000 spontaneous DNA lesions per day (Lindahl, 1993). As SCs ensure the lifetime maintenance of a given tissue, any misrepair of DNA damage can be transmitted to their differentiated daughter cells, thereby compromising tissue integrity and function. Consequently, mutations that diminish the renewal and/or differentiation potential of SCs can result in tissue atrophy and aging phenotypes, whereas mutations providing a selective advantage to the mutated cells can lead to cancer development (Rossi et al., 2008).

As such, a delicate balance must be struck to prevent exhaustion and transformation of the SC pool while maintaining the ability of SCs to preserve homeostasis and to respond to injury when necessary. To fulfill these demands, the numbers of SCs and their functional quality must be strictly controlled through a balance of cell-fate decisions (self-renewal, differentiation, migration, or death), which are mediated by a complex network of cell-intrinsic regulation and environmental cues (He et al., 2009; Weissman, 2000). Specific protective mechanisms also ensure that SC genomic integrity is well preserved and 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). Altogether, these attributes of SCs ensure tissue maintenance and function throughout the lifetime of an organism, while limiting atrophy and cancer development.

DNA-Damage Response

All living cells, including tissue-specific SCs, must constantly contend with DNA damage (Sancar et al., 2004) (Figure 1). Due to its chemical structure, DNA is particularly sensitive to spontaneous hydrolysis reactions which create abasic sites and base deamination. Furthermore, ongoing cellular metabolism generates ROS and their highly reactive intermediate metabolites, which can create 8-oxoguanine lesions in DNA as well as a variety of base oxidations and DNA strand breaks that are all highly mutagenic and can lead to genomic instability. DNA is also constantly assaulted by mutagens present in the external environment. UV light from the sun, as well as 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.

Consistent with the wide diversity of potential DNA lesions, eukaryotic cells exhibit many highly conserved DNA repair mechanisms that can recognize and repair different types of DNA damage with varying fidelity and mutagenic consequences (Lombard et al., 2005) (Figure 1). For instance, base modifications induced by spontaneous chemical reactions and ROS-mediated DNA lesions are repaired by base excision repair (BER), whereas nucleotide modifications induced by chemicals and UV light are repaired by the nucleotide excision repair (NER) pathway. The pathways that mediate the repair of DSBs vary depending on the cell-cycle status of the damaged cells. During the G_0/G_1 phase, DSBs are repaired by the nonhomologous end-joining (NHEJ) pathway, while, during the S-G_2/M

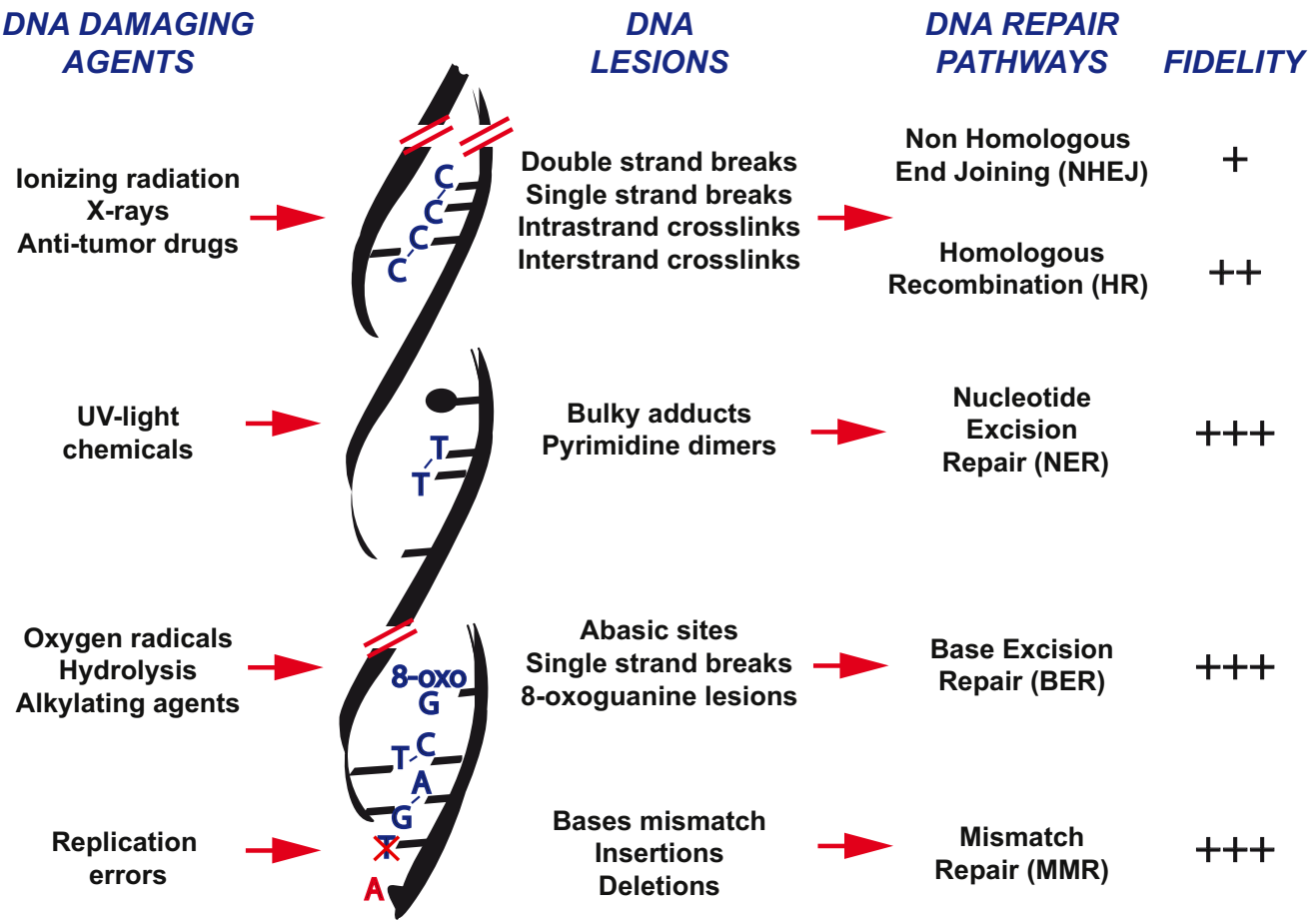

Figure 1. DNA-Repair Pathways in Mammalian Cells
Each type of DNA assault results in a different type of lesion, which can be repaired with different fidelity by distinct and highly specialized repair pathways.

phase, these lesions are repaired by the homologous recombination (HR) pathway. These two modes of DNA repair are not equally faithful. HR is an error-free DNA repair mechanism due to the use of the other intact strand as a template, while NHEJ is an error-prone repair mechanism, which may result in small deletions, insertions, nucleotide changes, or chromosomal translocations due to the absence of an intact template for repair. Lastly, replication errors leading to insertion, deletion, and base misincorporation resulting in base mispairing are corrected by the mismatch repair (MMR) pathway.

Irrespective of the type of lesion and the repair mechanism, DNA damage is rapidly sensed and activates evolutionarily conserved signaling pathways, known collectively as the DNA-damage response (DDR), whose components can be separated into four functional groups: damage sensors, signal transducers, repair effectors, and arrest or death effectors (Sancar et al., 2004) (Figure 2). Ultimately, activation of DDR leads to the phosphorylation and stabilization of p53, inducing its nuclear accumulation and upregulation of its target genes (d'Adda di Fagagna, 2008). Depending upon the extent of DNA damage, the type of cell undergoing DNA damage, the rapidity of DNA repair, the stage of the cell cycle, the strength and the duration of p53 activation, and the genes transactivated by p53, cells can either undergo transient cell-cycle arrest (through induction of the cyclin-dependant kinase inhibitor *p21*), programmed cell death (through induction of the pro-apototic *bcl2* gene family members *bax*, *puma* and *noxa*), or senescence (through induction of the cyclin-dependant kinase inhibitor *p16/Ink4a* and the tumor suppressor gene *p19/ARF*).

Diversity of DNA Repair Mechanisms in Tissue-Specific Stem Cells

The critical role of the different DNA repair mechanisms for overall tissue integrity and function is well illustrated by the severe clinical consequences observed in both humans and mice for mutations in genes regulating these pathways (Hakem, 2008). The involvement of tissue-specific SCs in mediating such symptoms and the role of the diverse DNA-damage recognition and DNA-repair mechanisms in maintaining tissue-specific SC function is now starting to emerge (Kenyon and Gerson, 2007).

Defects in DSB recognition machinery lead to premature aging, neurodegeneration, and increased cancer susceptibility. ATM (ataxia-telengiectasia mutated), ATR (ATM and Rad3 related), and DNA-PKs are DNA-damage-sensing protein kinases that, through a series of phosphorylation events, signal the presence of DNA lesions and initiate DNA repair or cell-cycle

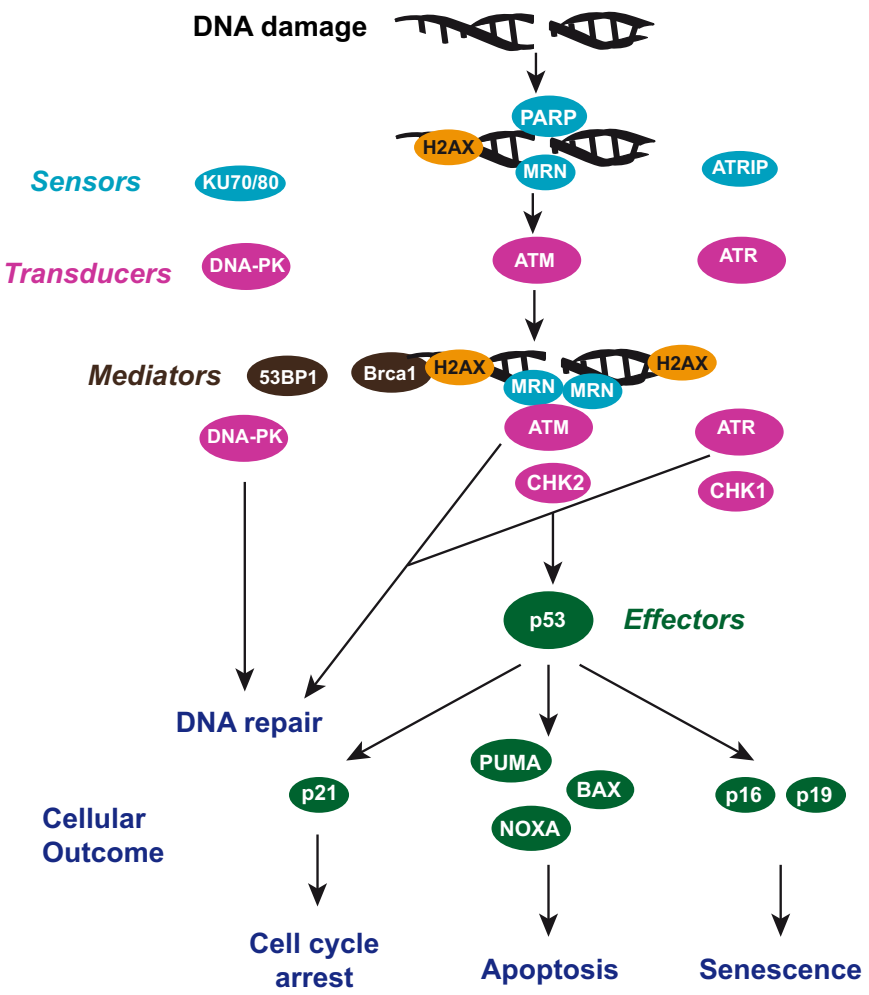

Figure 2. DNA-Damage Response Pathways

Upon DNA damage, distinct factors detect, transmit, and amplify the DNA-damage signal. DNA double-strand breaks can be repaired by homologous recombination (mediated among other factors by the MRN complex, ATM, and Brca1) or by nonhomologous end-joining (in which the Ku70/Ku80/DNA-PKcs complex plays a major role). This DNA-damage response converges upon p53 which, depending on the target genes activated, regulates different cellular outcomes.

arrest (Figure 2). Patients with mutations in ATM present blood vessel abnormalities, cerebelar degeneration, immunodeficiency, and increased risk of cancers (Hoeijmakers, 2009). Mice lacking *Atm*, like ATM patients, are extremely sensitive to IR exposure and have decreased somatic growth, neurological abnormalities, decreased T cell numbers, and exhibit premature hair graying and infertility (Barlow et al., 1996). Many of these phenotypes can be linked to defects in SC function, which highlights the critical role of this DDR component for the survival and preservation of various SC compartments. *Atm*-deficient hematopoietic SCs (HSCs) harbor increased ROS levels and display an overall decrease in number and function over time, leading to eventual hematopoietic failure (Ito et al., 2004, 2006). *Atm* deficiency also sensitizes mice to IR-induced premature melanocyte SC differentiation, resulting in hair graying (Inomata et al., 2009). Germ cell development is also altered in *Atm*-deficient mice, and mutant animals experience a progressive loss in germ SCs (spermatogonia) and become infertile (Takubo et al., 2008). Mutations in ATR also cause 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). The MRE11, RAD50, and NBS1 (MRN) complex senses DSBs, unwinds the damaged region of DNA, serves as part of the repair scaffolding, and induces downstream signaling including ATM activation (Figure 2). 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). Moreover, mutations in *BRCA1* and *BRCA2*, two DSB mediators that trigger DNA repair through the HR pathway (Figure 2), 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 SCs (Liu et al., 2008).

While no spontaneous mutations in NHEJ pathway components have been reported so far in human syndromes associated with premature aging or increased risk of cancers, the inactivation of various NHEJ genes in mice has demonstrated their essential function in lymphocyte development and prevention of lymphoma. The core components of the NHEJ repair pathway include the end-binding and end-processing proteins Ku70, Ku80, DNA-PKcs, and Artemis, as well as the ligation complexes XRCC4, LigIV, and Cerrunos (Lombard et al., 2005). As NHEJ is critical for V(D)J recombination during lymphocyte maturation, many of the mutant mouse models deficient in particular NHEJ components exhibit arrested lymphoid development. Mice carrying a Lig4^{y288c} hypomorphic mutation also display growth retardation, immunodeficiency, and pancytopenia associated with severe HSC defects (Kenyon and Gerson, 2007; Nijnik et al., 2007). Mice lacking the end-binding and end-processing components of NHEJ, Ku70, and Ku80 have stress-induced HSC self-renewal defects associated with poor transplantability,

increased apoptosis, decreased proliferation, and impaired lineage differentiation (Kenyon and Gerson, 2007; Rossi et al., 2007).

Mutations in NER pathway components induce human syndromes known as Xeroderma Pigmentosum (XP), Cockayne syndrome (CS), and Trichothiodistrophy (TTD), which are characterized by premature aging, neurodegeneration, and extreme photosensitivity, especially in XP syndromes (Hoeijmakers, 2009). XP patients often completely lack NER repair activity and have increased incidence of skin cancer, while CS and TTD patients have defects in transcription-coupled repair, which has little mutagenic effect because it only deals with lesions in the transcribed strand. Mice expressing XPD^{TTD}, a mutated form of an essential NER component, have decreased HSC function with reduced self-renewal potential and increased apoptosis levels (Rossi et al., 2007). Mice deficient in *Ercc1*, a component of both NER and intrastrand crosslink (ICL) repair, die within 4 weeks of birth, have multilineage hematopoietic cytopenia due to progenitor depletion, HSC senescence, and a defective response to DNA crosslinking by mitomycin C (Hasty et al., 2003; Prasher et al., 2005).

Mutations in MMR pathway components induce hereditary nonpolyposis human colorectal cancer known as Lynch syndrome, which presents with about an 80% lifetime risk of developing colorectal cancers as well as other malignancies (Hoeijmakers, 2009). Mice mutant for genes important for the MMR pathway, including *Msh2* and *Mlh1*, also display higher frequencies of hematological, skin, and gastrointestinal tumors, consistent with a critical role of the MMR in preventing accumulations of oncogenic mutations (Hakem, 2008). In addition, mice lacking *Msh2* exhibit defective HSC activity, with enhanced microsatellite instability observed in their progeny (Reese et al., 2003).

Other human conditions associated with defects in DNA-damage recognition and repair pathways include Fanconi's Anemia (genetic defects in the FANC family of proteins), Bloom's or Werner's syndromes (both caused by mutations in DNA helicases), and a range of diseases associated with telomerase dysfunction and telomere instability (Kenyon and Gerson, 2007). These diseases are not specifically reviewed here, but their complex pathologies involve defects in various tissue-specific SCs.

DNA-Damage Response in Tissue-Specific SCs

While tissue-specific SCs share the same purpose of maintaining organ functionality, recent studies have shown that the mechanisms of their responses to DNA damage, the outcome of their DDR, and the consequences of DNA repair for their genomic stability vary greatly between tissues.

Hematopoietic SCs

The hematopoietic (blood) system is one of the best-studied adult tissues in terms of its hierarchical development, in that all blood cell lineages derive from a small number of quiescent HSCs via a highly proliferative amplifying progenitor compartment (Orkin and Zon, 2008). Being a highly regenerative compartment, it is also one of the most radiosensitive tissues in the body (<4 Gy), and one of the first organ systems to fail after total body irradiation. IR exposure differentially affects hematopoietic cells depending on their state of maturity, with HSCs being more radioresistant than their downstream progeny (Meijne et al., 1991). By comparing the way HSCs and their differentiated progeny respond to low doses of IR (2 to 3 Gy), recent work has begun to clarify the ways in which HSCs at different stages of ontogeny deal with DNA damage and the mutagenic consequences of different DNA repair mechanisms in this tissue-specific SC population (Figure 3A).

HSCs are specified in the aorta-gonad-mesonephros (AGM) region of the developing fetus, are actively expanded in several anatomic locations, including the liver and placenta, during fetal development, and are finally seeded in the bone marrow cavity during late embryogenesis. In the bone marrow, HSCs progressively mature after birth to become the quiescent adult HSCs that are maintained during the lifetime of the organism. Fetal and adult HSCs differ in many aspects of their biological regulation, including cell-cycle status and transcriptional control (Orkin and Zon, 2008). Using human umbilical cord blood (CB)-derived HSCs, which are highly proliferative, circulating cells that are still considered to be of fetal origin, Milyavsky and colleagues found that irradiated (3 Gy) CB-derived HSCs had a slower rate of DSB repair than more mature progenitors and increased levels of apoptosis mediated in part through the ASPP1 protein, which could be reversed if *p53* expression was silenced or *bcl2* expression was enhanced (Milyavsky et al., 2010). Upon primary transplantation, irradiated CB-derived HSCs could not successfully engraft into immunodeficient mice. In contrast, irradiated cells with disabled *p53* or *bcl2* overexpression could be serially transplanted, albeit with decreased efficiency compared to nonirradiated normal cells. In this context, transplanted CB-derived HSCs with disabled *p53* reconstituted even less well than cells with *bcl2* overexpression, and their progeny harbored high levels of DSBs that were not observed in the progeny of *bcl2* overexpressing cells. This study emphasizes the role of p53-mediated DDR and the Bcl2 family of prosurvival genes in HSC function (Asai et al., 2010; Seita et al., 2010; Weissman, 2000), and indicates that the main outcome of the DDR in fetal HSCs is induction of apoptosis and overt cell elimination (Figure 3A). On the other hand, using adult mouse HSCs that are kept mostly quiescent within the bone marrow cavity, Mohrin and colleagues showed a very different response to irradiation, with overt cell survival and DNA repair being the main outcomes of the DDR (Mohrin et al., 2010). Adult HSCs, either quiescent or induced to proliferate by cytokine pretreatment, engage specialized response mechanisms that protect them from low doses of IR (2 Gy). In quiescent HSCs, these mechanisms include enhanced prosurvival gene expression (*bcl2*, *bcl-xl*, *mcl1*, *a1*), which inhibits cell death induced by p53 proapototic genes (*bax*, *noxa*, *puma*), likely allowing p53-mediated induction of *p21* to engage a transient growth-arrest response and to permit DNA repair. While the exact mechanism of the survival response in proliferating HSCs is less clear, they were found to be as radioresistant as quiescent HSCs (Mohrin et al., 2010). Dictated by their cell-cycle status, proliferating HSCs use the high-fidelity HR pathway to repair DSBs, while quiescent HSCs employ the error-prone NHEJ pathway. Irradiated quiescent HSCs display high levels of chromosomal abnormalities when compared to proliferating HSCs, and their progeny show persistent genomic instability associated with misrepaired DNA and engraftment defects in secondary recipient mice. Since NHEJ appears to

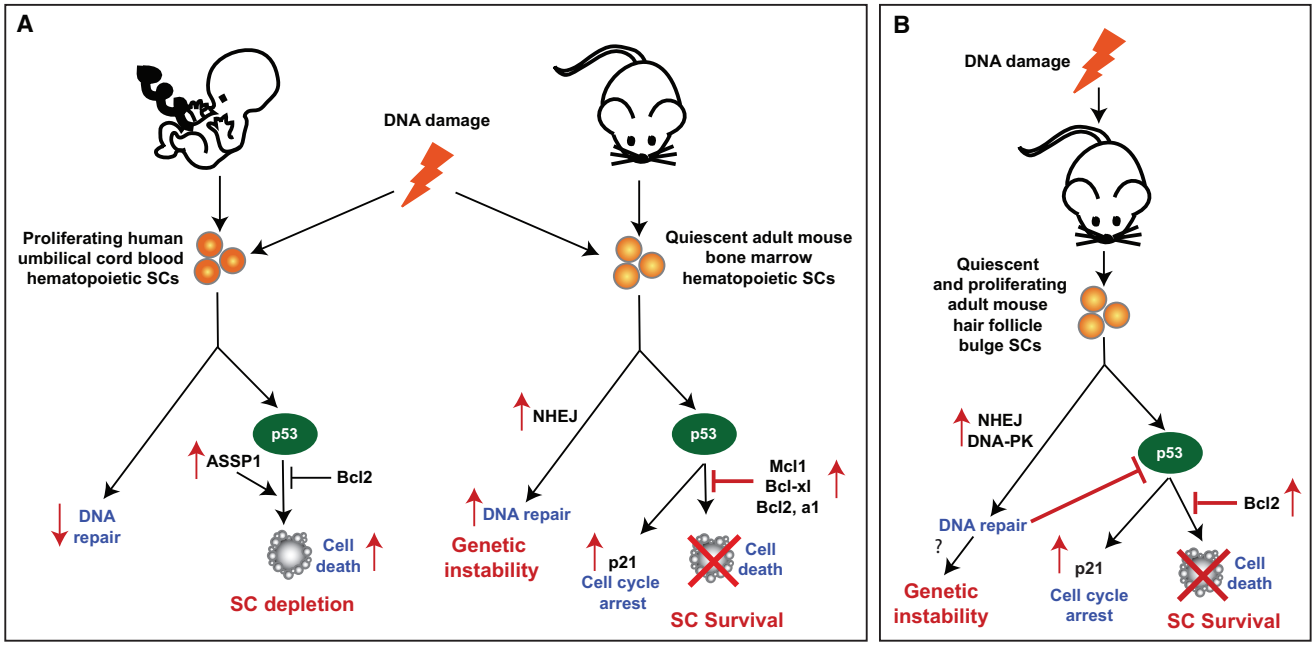

Figure 3. DNA-Damage Response in Hematopoietic and Hair Follicle Bulge Stem Cells

(A) Human umbilical cord blood-derived HSCs and mouse bone marrow-derived HSCs exhibit opposite outcomes following irradiation-induced DNA damage, with different consequences for their overall maintenance and genomic integrity.

(B) Upon irradiation, mouse hair follicle bulge stem cells exhibit transient p53 activation due, in part, to high levels of DNA-PK-mediated NHEJ repair and higher Bcl2 expression that block apoptosis, resulting in enhanced survival.

be the initial and most commonly used DNA repair mechanism in quiescent HSCs, these results help explain why most mouse models lacking functional components of DSB recognition and repair pathways undergo hematopoietic failure upon genotoxic stress (Hakem, 2008). Moreover, this study indicates that while adult HSCs, in contrast to fetal HSCs, may survive DNA-damaging insults, they do not emerge unscathed (Figure 3A), which might have direct implications for aging and cancer development. It may also explain why cancer patients treated with radiotherapy or chemotherapy may develop leukemias and lymphomas (blood cancer) or myelodysplasias (bone marrow failure) because the use of error-prone DNA repair in quiescent HSCs may be at the heart of these dangerous side effects of cancer treatment.

Taken together, these two studies (Milyavsky et al., 2010; Mohrin et al., 2010) unveil some striking differences in the outcome of irradiation-induced DDR in HSCs from different species and at different developmental stages. While it is possible that different organisms with vastly different lifespans have evolved distinct strategies to cope with DNA damage, it is tempting to speculate that these differences reflect an adaptation in the stress response mechanisms used by HSCs at distinct stages of ontogeny to ensure optimal function of the blood system. During embryogenesis and until birth, the goal is to expand the SC population while protecting its genomic integrity in order to establish a pool of pristine HSCs that will ensure blood homeostasis for the lifetime of the organism. In this context, the efficient elimination of irradiated human CB-derived HSCs described by Milyavsky and colleagues fulfill this demand by eliminating damaged fetal HSCs that could be detrimental to the organism and its reproductive purpose. Conversely, in adults, the main function of the HSC compartment is to preserve blood homeostasis and to quickly respond to hematopoietic needs (blood loss, infection, etc.). The fact that adult HSCs reside in hypoxic niches in the BM cavity and are mostly kept in a quiescent phase of the cell cycle contribute to their overall maintenance (self-renewal) and protect their genomic integrity (fitness) by minimizing DNA damage associated with ROS production, cellular respiration, and cell division (Orford and Scadden, 2008; Rossi et al., 2007). In this context, the survival and efficient DNA repair of irradiated mouse adult HSCs described by Mohrin and colleagues fulfills the same purpose by protecting the most important cells of the tissue. Since both quiescent and proliferating mouse adult HSCs show similar radioresistance, it is likely that the radiosensitivity displayed by human CB-derived HSCs reflect cell-intrinsic differences in transcriptional programs or chromatin states between HSCs at various stages of development. Additional investigations are clearly needed to fully understand the mechanisms underlying these differences in DDR outcomes between fetal and adult HSCs.

However, the short-term survival strategy used by adult HSCs likely comes at a cost for their long-term genomic integrity. While quiescence is one of the very mechanisms that protects adult HSC function, it also renders damaged HSCs intrinsically vulnerable to mutagenesis because it forces them to use the error-prone NHEJ pathway to repair DSBs, thereby increasing the risk of creating mutations in this self-renewing population. In fact, the accrual of chromosomal translocations resulting from unfaithful DNA repair following DSBs is a hallmark of human blood malignancies (Look, 1997). Such accumulation over time of NHEJ-mediated mutations may hinder cellular performance

and could be a major contributor to the loss-of-function occurring with age in the HSC compartment and to the development of age-related hematological disorders (Rossi et al., 2007).

Epidermal SCs

The skin epidermis is composed by the juxtaposition of the many pilosebaceous units consisting of a hair follicle, its associated sebaceous gland, and its surrounding interfollicular epidermis. Different classes of SCs ensure homeostasis of the skin epidermis (Blanpain and Fuchs, 2009). Multipotent hair follicle bulge SCs (BSCs) contribute to the cyclic regeneration of the hair follicle and to the repair of the interfollicular epidermis following wounding. In the absence of injury, the interfollicular epidermis can self-renew independently of BSCs through the presence of unipotent progenitors scattered throughout the basal region of the epidermis. Specialized SCs and progenitor cells are also found in the infundibulum and sebaceous glands (Blanpain and Fuchs, 2009).

Since the epidermis serves as a barrier between the body and the external environment, it is constantly assaulted by genotoxic stress such as UV irradiation. As discussed earlier, UV radiation causes the formation of thymidine dimers, (6-4) pyrimidine photoproducts, and ROS-induced DNA lesions that are repaired by the NER, NHEJ, or HR pathways, depending on the type of damage and the state of the cell cycle. Upon UV irradiation, basal epidermal cells exhibit sustained p53 activation compared to the more differentiated suprabasal cells (Finlan et al., 2006). Following chronic administration of UV radiation, slow-cycling SCs and progenitor cells of the infundibulum and sebaceous glands also retain UV-induced photoproducts longer than more differentiated cells of the epidermis, suggesting a decrease in the repair activity of these cells (Nijhof et al., 2007). Recently, *Nrf2* has been shown to regulate the expression of critical regulators of oxidative stress (such as several enzymes of the glutathione metabolism) and to protect the epidermis from UV-induced apoptosis. The gradient of apoptosis levels observed between basal (high) and suprabasal (low) cells following UV irradiation is inversely correlated with *Nrf2* expression. Surprisingly, while Nrf2 overexpression protects basal cells from UV induced apoptosis, it does not decrease the proportion of cells that harbor thymidine dimers. In addition, suprabasal expression of *Nrf2* offers some protection from UV-induced apoptosis to basal cells through a paracrine mechanism (Schafer et al., 2010). These data indicate that proliferative cells of the interfollicular epidermis are more sensitive to UV-mediated apoptosis relative to their more committed progeny.

While the skin epidermis is more radioresistant than the blood system, acute administration of more than 5 Gy results in severe skin reactions consisting of inflammation (erythema) and loss of differentiated skin layers (desquamation) that rapidly appear following IR, whereas hair loss and chronic ulcerations appear with a delay of 2 to 3 weeks after IR administration. The sensitivity of the epidermis to IR is also illustrated by the common side effects of radiotherapy, which include acute and chronic dermatitis and an increased incidence of skin cancer (Goldschmidt and Sherwin, 1980). While the field is still in search of specific cell-surface markers that will allow high purity isolation of interfollicular epidermal progenitors, a combination of markers, including α6 integrin and CD71, have been used to enrich SCs from the mouse and human interfollicular epidermis (Li et al., 1998; Tani et al., 2000). Following exposure to low doses of IR, rapidly cycling human epidermal progenitor cells ($\alpha6H^{hi}/CD71^{+}$) undergo apoptosis and display decreased in vitro colony forming efficiency, whereas slow-cycling human epidermal SCs ($\alpha6H/CD71^{-}$) were resistant to IR-induced cell death (Rachidi et al., 2007). The enhanced survival of human epidermal SCs upon IR exposure has been linked to a higher secretion of FGF2 following DNA damage, which increases DNA repair activity in epidermal SC by autocrine/paracrine mechanisms (Harfouche et al., 2010). While these studies have been performed ex vivo, Sotiropoulou and colleagues have recently investigated how epidermal cells respond to DNA damage within their native niche and showed that multipotent hair follicle BSCs, like HSCs, are more resistant to DNA-damage-induced cell death compared to the other cells of the epidermis (Sotiropoulou et al., 2010). At least two important mechanisms contribute to the higher resistance of BSCs to IR-mediated DNA damage (Figure 3B), both which are independent of the relative quiescence of these cells and of the induction of premature senescence. First, BSCs express higher levels of the antiapoptotic protein Bcl2, and the proportion of BSCs undergoing apoptosis is increased in *bcl2* null mice, demonstrating that similar to HSCs, a higher expression of prosurvival factors contributes to the resistance of BSCs to apoptosis. The other contributing mechanism is the transient nature of DDR activation in BSCs. Soon after IR exposure, p53 is expressed in the nuclei of almost all epidermal cells, including BSCs, and is required for DNA-damage-induced cell death in the epidermis (Botchkarev et al., 2000; Song and Lambert, 1999; Sotiropoulou et al., 2010). However, unlike other cells of the epidermis, the number of BSCs expressing p53 is greatly decreased by 24 hr following irradiation, and mutant mice exhibiting sustained expression of p53 show increased IR-induced apoptosis in BSCs. This indicates that the short duration of IR-mediated p53 activation promotes BSC survival following DNA damage. Interestingly, BSCs also display accelerated DNA repair and enhanced NHEJ repair activity. In SCID mice, which have a mutation in *DNA-PK* and thus exhibit decreased NHEJ activity, BSCs are radiosensitive, suggesting that accelerated NHEJ-mediated DSB repair contributes to their protection against IR exposure. The importance of DDR in BSCs is also illustrated by the SC exhaustion and progressive alopecia that occurs in mice where *Atr* has been deleted in hair follicle BSCs and their progeny (Ruzankina et al., 2007).

Because NHEJ is an error-prone DNA repair mechanism, the higher resistance of BSCs to DNA-damage-induced apoptosis and the accelerated NHEJ-mediated DNA repair activity could be, like in HSCs, a double-edged sword that promotes short-term survival of BSCs at the expense of their long-term genomic integrity and could potentially allow for the accumulation of cancerous mutations (Figure 4). Consistent with this notion, SCID mice and mice deficient for Bcl-X_L, a prosurvival gene, show decreased susceptibility to chemical carcinogenesis (Kemp et al., 1999; Kim et al., 2009), which has been attributed to the elimination of mutated BSCs by apoptosis.

Melanocyte SCs

Melanocytes are neural crest-derived cells responsible for the pigmentation of skin and hair. The mature melanocytes responsible for hair color are derived from melanocyte SCs (MSCs),

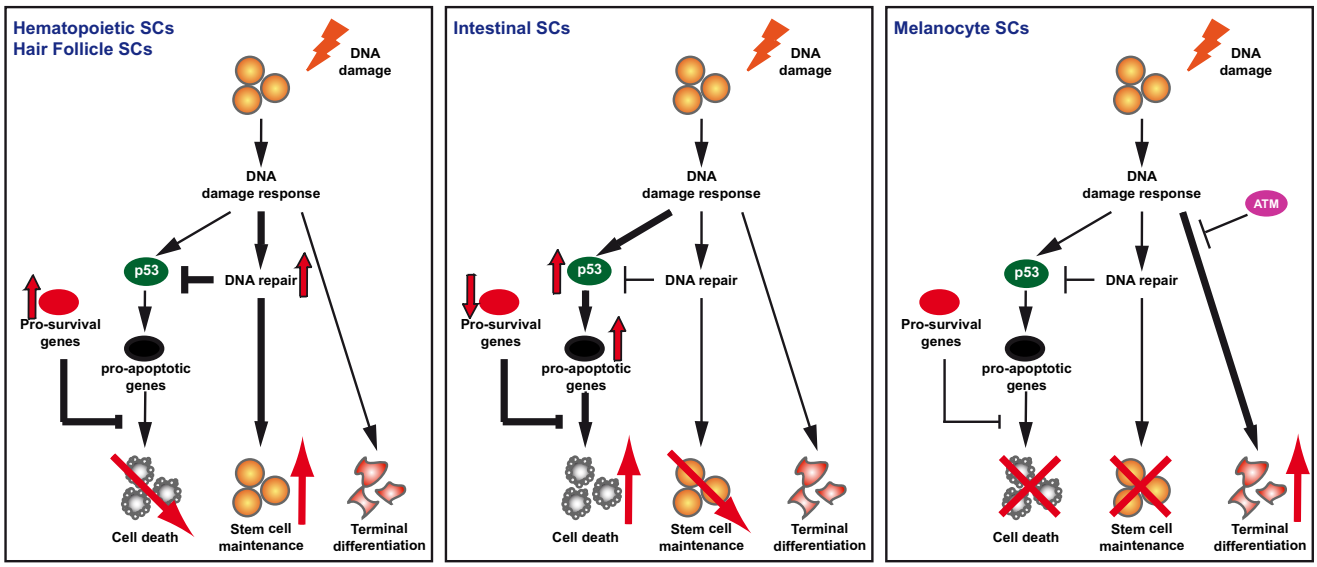

Figure 4. DNA-Damage Response in Tissue-Specific Stem Cells
Common and distinct pathways of DNA-damage response in different types of tissue-specific SCs.

which reside in the same niche as hair follicle BSCs. At each cycle of hair regeneration, MSCs are stimulated to proliferate and give rise to transit amplifying cells, which will expand in the lower hair follicle before undergoing terminal differentiation, which results in the integration of their pigment into the new hair. At the end of each hair cycle, mature melanocytes undergo apoptosis and are eliminated with the rest of the follicle, to be subsequently replenished by the renewal and differentiation of MSCs during the next cycle (Robinson and Fisher, 2009). Hair graying, which is one of the most common signs of aging, results from the depletion of MSCs from the hair follicle. The onset of hair graying in mice and humans is accompanied by the presence of ectopically pigmented melanocytes, suggesting premature differentiation of MSCs within their niche (Nishimura et al., 2005). Premature hair graying can also result from a hypomorphic mutation in *Mitf*, the main regulator of MSC differentiation, that results in a downregulation of *bcl2* and in premature differentiation of MSCs in the hair follicle (McGill et al., 2002). Bcl2 is critical for MSC maintenance as *bcl2* null mice lose their coat pigmentation after the first hair cycle due to massive MSC apoptosis (Nishimura et al., 2005). Premature hair graying and progressive MSC loss also occur following administration of DNA damaging agents such as IR, mitomycin C, or hydrogen peroxide (Inomata et al., 2009). While the mechanisms underlying the DDR in MSCs are not yet fully understood, p53, p16, and $p19^{ARF}$, although transiently activated by DNA damage, are not responsible for the premature differentiation and loss of MSCs. Indeed, mice deficient for *p53* or the *Ink4a* locus (p16 and $p19^{ARF}$) are not protected from DNA-damage-induced hair graying, contrasting with the requirement of p53 in mediating DNA-damage-induced cell death in other tissue-specific SCs. In contrast, DNA damage induces prolonged activation of the canonical differentiation program of MSCs, including sustained upregulation of *Mitf*, a key regulator of melanocyte differentiation and melanogenic enzymes, which in turn stimulates the premature and ectopic differentiation of MSCs within their niche. The ATM checkpoint regulator also exerts a protective function in MSCs because *Atm* null mice and ATM-deficient patients exhibit premature hair graying (Hakem, 2008) and loss of *Atm* sensitizes mice to IR-induced premature MSC differentiation (Inomata et al., 2009).

Despite being located in the same hair follicle niche, BSCs and MSCs respond very differently to DNA damage. Both types of SCs do not senesce or commit apoptosis upon DNA damage, but while BSCs repair their DNA rapidly and express high levels of antiapoptotic molecules in order to avoid programmed cell death, MSCs are eliminated by premature differentiation (Figure 4). These different outcomes imply that cell intrinsic properties are more important than the local microenvironment in controlling DDR in skin SCs. It is interesting to note that melanoma, a malignant tumor of melanocytes, does not arise from hair follicle MSCs but rather from skin melanocytes. These cells are located along the interfollicular epidermis, suggesting that the premature differentiation of MSCs following DNA damage may serve to eliminate precancerous MSCs residing in the hair follicle.

Intestinal SCs

The intestinal tissue is very sensitive to DNA damage. Acute whole-body irradiation (<6 Gy) induces considerable damage to the intestine, resulting in severe diarrhea and electrolyte imbalances, which can be lethal in extreme cases. The intestinal lining is a simple epithelium composed of a single layer of cells that can be divided into two compartments: the proliferative base of the intestine, called the crypt, and the differentiated intestinal cells forming the villi that face the intestinal lumen. The intestinal SCs (ISCs) are localized at the bottom of the crypt, where they proliferate to give rise to transit amplifying cells, which are found along the crypt, and divide faster and migrate to the upper part of the crypt where they undergo cell-cycle arrest and terminal differentiation (Barker et al., 2010; Casali and Batlle, 2009; Marshman et al., 2002). Although the exact

position of the ISCs within the crypt is still under intense debate, it has long been suggested that ISCs reside at the +4 position from the base of the crypts and that these SCs are more quiescent compared to the other crypt cells. Consistent with that notion, Bmi1, which is preferentially expressed in +4 crypt cells, induced long-term labeling of the crypto-vilus unit in *Bmi1CRE*^{ER} reporter mice, consistent with the labeling of long-lived multipotent ISCs (Sangiorgi and Capecchi, 2008). A second population of ISCs expressing Lgr5, a leucine-rich orphan G protein-coupled receptor and Wnt pathway activated gene, has recently been identified (Barker et al., 2007). Lgr5^{+} cells cycle more frequently than the +4 cells and are located at the bottom of the crypt intercalated between the paneth cells. Lineage tracing experiments using *Lgr5-GFP-IRES-Cre-ERT;;RosaLacZ* reporter mice demonstrated that Lgr5^{+} cells give rise to all intestinal cell lineages and result in the long-term labeling of the cryptovilus unit, also consistent with the labeling of long-lived multipotent ISCs.

ISCs are extremely sensitive to DNA damage and undergo massive apoptosis upon low doses of irradiation (1 Gy). Interestingly, while it is generally assumed that radiosensitivity is correlated with cell-cycle status (Gudkov and Komarova, 2003), the apoptosis sensitivity of intestinal crypt cells is inversely correlated with their relative quiescence. The most quiescent ISCs located at +4 position are the most sensitive to IR-induced cell death, followed by the more active Lgr5^{+} ISCs, whereas the rapidly cycling transit-amplifying cells appear to be the most radioresistant (Barker et al., 2007; Potten et al., 2002; Wilson et al., 1998). Different mechanisms are responsible for the extreme sensitivity of ISCs to DNA damage, including an enhanced activation of the p53 pathway, lower expression of the antiapoptotic protein Bcl2 (Merritt et al., 1995), and general lack of DNA repair activity (Potten, 2004). Upon irradiation, expression of p53 and its downstream target genes *p21* and *puma* increases throughout the crypts, but the frequency of p53-positive cells and the levels of expression of its target genes are higher at the base of the crypt and progressively decrease along the crypts toward the vilus (Merritt et al., 1994; Qiu et al., 2008; Wilson et al., 1998). Furthermore, IR does not induce apoptosis in the intestine of *p53* null mice (Merritt et al., 1994; Qiu et al., 2008; Wilson et al., 1998). IR-induced ISC apoptosis is also blocked in *puma*-deficient mice, and ISC survival is prolonged after administration of *puma* antisense nucleotides, thereby demonstrating that Puma is the main proapoptotic target of the p53-mediated DDR in ISCs (Qiu et al., 2008). In contrast to other SC populations described above, *bcl2* expression is not detected in ISCs and irradiated *bcl2* null mice only show a modest increase in ISC apoptosis, suggesting that Bcl2 does not play a critical role in protecting ISCs from DNA-damage-induced cell death (Merritt et al., 1995). Finally, the absence of an irradiation dose response of crypt degeneration suggests that quiescent ISCs lack DNA repair capacity, thereby increasing their propensity to undergo apoptosis following DNA damage (Hendry et al., 1982; Potten, 2004).

The architecture of the colon resembles that of the small intestine. Similar to ISCs, colonic SCs (CoSCs) are also localized at the bottom of the crypt and express Lgr5, although CoSCs exhibit a longer cell-cycle time than ISCs. Interestingly, the DDR of CoSCs differs significantly from that of ISCs, with CoSCs being considerably more radioresistant than ISCs (Figure 4). It is estimated that CoSCs require eight times the dose of irradiation needed by ISCs to reach similar levels of apoptosis (Barker et al., 2007; Potten and Grant, 1998; Pritchard et al., 2000). The greater radioresistance of CoSCs has been attributed to a lower expression of *p53* (Hendry et al., 1997; Merritt et al., 1994) and higher expression of *bcl2* (Merritt et al., 1995; Qiu et al., 2008). Furthermore, in contrast to ISCs, CoSCs from *bcl2* null mice show a much greater increase in DNA-damage-induced apoptosis, demonstrating that *bcl2* expression in CoSCs does contribute to their higher relative radioresistance. The altruistic suicide of ISCs in response to DNA damage could decrease the acquisition of precancerous mutations in these cells and potentially explain the rarity of intestinal neoplasia compared to the higher frequency of colonic cancers, despite the higher cellular turnover of the intestine.

Germline SCs

Primordial germ cells (PGCs) are transient precursors of germ SCs (GSCs), which upon meiosis give rise to the gametes (sperm and egg), which are the only cells capable of transferring genetic information from one generation to the next (Chuva de Sousa Lopes and Roelen, 2010; Laird et al., 2008; Richardson and Lehmann, 2010). PGCs are specified in the embryo, migrate to the gonadal ridges were they undergo sex determination, and give rise to the female (oogonia) or the male (spermatogonia) GSCs. The spermatogonia exhibit an almost unlimited life span, remaining quiescent until puberty, at which point they re-acquire the ability to self-renew, undergo meiosis, and produce mature male gametes for the lifetime of the organism. In sharp contrast, the pool of oogonia is established during embryogenesis, and consequently, females are born with a finite number of oogonia.

The generation of haploid chromosomes during meiosis requires many of the proteins involved in DNA repair (Sasaki et al., 2010). During PGC maturation, genome-wide DNA demethylation occurs in order to erase genomic imprinting. DNA demethylation in mouse PGCs is initiated by the appearance of single-strand breaks and activation of the BER pathway, which may be linked to deamination of methylcytosine or to other yet-to-be-discovered mechanisms (Hajkova et al., 2010). Mutations in the germ line can be extremely dangerous and can either directly lead to sterility (Loft et al., 2003) or transmission of heritable genetic diseases by the gametes. Genetic aberrations in GSCs may occur upon radiation exposure, such as radiotherapy and radiological examination, or after exposure to teratogenic or mutagenic chemicals, but the main source of DNA damage is their normal metabolic activity and ROS production (Kujjo et al., 2010). Microarray analysis uncovered that DNA-damage sensors and multiple components of the NHEJ, BER, NER, and MMR pathways are expressed in human oocytes (Menezo et al., 2007), with a similar high expression of DNA repair proteins found in human sperm (Galetzka et al., 2007), which suggest that GSCs and gametes are well equipped to respond to DNA damage. Accordingly, spermatogonia in *Atm*-deficient mice are progressively lost, undergo meiotic arrest, accumulate DNA damage, and lose their self-renewal potential in a *p21*-dependent manner (Takubo et al., 2008). Mice expressing the hypomorphic mutation of Rad50^{k22m} also show severe attrition of spermatogonia, which could be minimized by loss of p53 (Bender et al., 2002).

The cell-cycle duration of human spermatogonia is estimated to be around 16 days, with male GSCs being mostly kept in the G_0/G_1 phase of the cell cycle. Consequently, NHEJ is the first line of DNA repair in these cells. Interestingly, in vitro studies in mice showed that spermatogonia are more sensitive to IR when they are quiescent than when they are proliferating (Forand et al., 2009; Moreno et al., 2001). In oogonia, the homologous chromosomes are close to each other and female GSCs preferentially repair their DNA using HR (Baker, 1971). Mutations in the HR repair pathway render female GSCs more susceptible to DNA-damage-mediated cell death as shown by the increase sensitivity to doxorubicin-induced apoptosis in oocytes from mice deficient in *Rad51* (Kujjo et al., 2010). Contrary to most SC populations and somatic cells, the DDR in female GSCs does not depend on p53. Instead, TAp63, an isoform of the p63 gene and a p53 homolog, is constitutively expressed in oocytes and is rapidly phosphorylated following DNA damage. Deletion of *TAp63* in mice results in a major increase in oocyte radioresistance, consistent with the notion that TAp63 is the primary mediator of DDR pathway in oocytes (Suh et al., 2006).

Mammary SCs

The mammary gland alternates between cycles of growth and degeneration in relation to the estrus cycle. Mammary stem cells (MaSCs) are responsible for homeostasis of the breast tissue and for the massive tissue expansion and remodeling that occurs during pregnancy and lactation (Visvader, 2009). MaSCs have been isolated from mice and humans and represent multipotent SCs that have the ability to self renew as well as to differentiate into ductal, alveolar, and myoepithelial cell lineages (Ginestier et al., 2007; Shackleton et al., 2006; Stingl et al., 2006). Breast cancer is the most common form of malignancies in women. Mutations in genes involved in DNA repair such as *BRCA1* and *BRCA2* are found in the majority of patients with hereditary breast cancers, demonstrating the importance of the HR-repair pathway in preventing the occurrence of mammary tumors (Bradley and Medina, 1998). Mice deficient for *Brca1* are embryonic lethal, but mice with a conditional deletion of *Brca1* in the mammary epithelium are viable, display severe abnormalities in mammary morphogenesis, and develop undifferentiated breast cancers (Hakem, 2008). Knockdown of *BRCA1* in human MaSCs leads to a decrease of differentiated luminal cells and an increase in cells with SC characteristics, which suggests that BRCA1 is required for normal MaSC differentiation and that *BRCA1* loss may result in the accumulation of genetically unstable MaSCs that are susceptible to cancer development (Liu et al., 2008).

While the role of DNA repair in mammary development, maintenance, and prevention of breast tumors is well established, the mechanisms underlying the DDR in MaSCs have only just begun to emerge. Mouse MaSCs are more radioresistant than their differentiated progeny, and their numbers increase following IR (Woodward et al., 2007). Interestingly, MaSCs present less DNA damage and rapidly activate the Wnt/β-catenin signaling pathway following IR. Furthermore, increasing β-catenin signaling by overexpression of Wnt1 or stabilized β-catenin increases the survival of MaSCs following DNA damage, indicating that Wnt/β-catenin signaling is an important component of the DDR in MaSCs that may promote MaSC survival through upregulation of survivin, a direct Wnt/β-catenin target gene (Chen et al., 2007; Woodward et al., 2007). It would certainly be interesting to determine whether the selective activation of Wnt/β-catenin pathway observed in MaSCs also occurs in other tissue-specific SCs and promotes their survival following DNA damage. Another mechanism that might promote MaSCs resistance to DNA damage is their low level of ROS compared their differentiated progeny (Diehn et al., 2009).

DNA-Damage Response in Cancer Stem Cells

A number of human cancers, including leukemia, glioblastoma, breast, and skin cancers, contain cells with higher clonogenic potential that are capable of reforming the parental tumors upon transplantation. These cells functionally resemble tissue-specific SCs, albeit with aberrant self-renewal and differentiation abilities, and have been collectively referred to as cancer SCs (CSCs), despite their variable developmental origin (Clarke and Fuller, 2006; Jordan et al., 2006). It has been suggested that CSCs are responsible for disease progression and tumor relapse after therapy. Recent studies indicate that CSCs may take advantage of the mechanisms of DNA repair used by tissue-specific SCs to mediate resistance to chemo- and radiotherapy.

CSCs in Leukemia

Leukemias are cancers of the blood system, which often arise due to deregulated HSC functions or acquisition of extended self-renewal capabilities by more mature progenitor cells (Passegue, 2005). Leukemia CSCs exist in acute myeloid leukemia (AML) and chronic myelogenous leukemia (CML) and have been shown to be more resistant to cancer therapies than the bulk of the leukemia cells, indicating that their survival may be responsible for disease persistence and cancer relapse (Elrick et al., 2005; Jordan et al., 2006). Leukemia CSCs also use to their advantage some protective mechanisms of HSCs, including quiescent cell-cycle status, localization to a hypoxic niche, and DDR mechanisms, to specifically escape chemo- and radiotherapy that kill the bulk of the tumor cells (Guzman and Jordan, 2009).

CML is a two-stage blood disease caused by the acquisition of the chromosomal translocation fusion product BCR/ABL in HSCs, which can be separated into chronic and acute phases. The transition from chronic to acute disease is still poorly understood, but the presence of DNA damage and the acquisition of additional chromosomal aberrations resulting in overall genomic instability in both HSCs and their downstream progeny is believed to play a critical role in this transition (Burke and Carroll, 2010). BCR/ABL expression increases intracellular ROS levels, which in turn enhances oxidative stress and DNA damage and deregulates DNA repair mechanisms, thereby promoting unfaithful and/or inefficient DNA repair leading to mutations and chromosomal aberrations (Perrotti et al., 2010). Malfunctioning MMR, mutagenic NER, and compromised DSB repair (both HR and NHEJ) are all hallmarks of cells expressing BCR/ABL (Burke and Carroll, 2010; Deutsch et al., 2001; Slupianek et al., 2002, 2006). Once DNA damage occurs, BCR/ABL-mediated signaling can also inhibit apoptosis, thereby allowing cells to survive DNA damage with which they normally would not be able to cope (Burke and Carroll, 2010; Deutsch et al., 2001; Slupianek et al., 2002, 2006). The 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). Indeed, evolution from HSC-derived CSCs to myeloid progenitor-derived CSCs has been observed during the transition to myeloid blast crisis in human CML and has been linked to activated mutations in the Wnt/β-catenin pathway and acquisition of aberrant self-renewal activity in HSC progeny (Rice and Jamieson, 2010). Preventing oxidative stress and correcting defects in DNA repair pathways in BCR/ABL-expressing CSCs at all stages of the disease may therefore be beneficial to limit the acquisition of drug resistance and slow down CML progression (Koptyra et al., 2006; Perrotti et al., 2010).

Leukemia CSCs maintain some of the same protective mechanisms as normal HSCs. CSCs in both CML and AML have been found to be quiescent (Elrick et al., 2005; Guan et al., 2003; Ishikawa et al., 2007), suggesting that cell-cycle restriction is one of the protective mechanisms that leukemia CSCs utilize to their advantage (Guzman and Jordan, 2009). Indeed, human AML CSCs transplanted into immunodeficient mice use quiescence as a protective mechanism against chemotherapy (Saito et al., 2010). When these cells are induced to exit quiescence and to enter the cell cycle by treating the mice with the cytokine G-CSF, AML CSCs become more sensitive to chemotherapy and are effectively eliminated in vivo. Leukemia CSCs are also able to co-opt other mechanisms used by normal HSCs for their protection, such as p53-mediated induction of *p21* and resulting growth arrest that has recently been found to be critical in protecting adult HSCs from IR (Mohrin et al., 2010). Expression of the PML/RAR or AML1/ETO fusion oncoproteins in murine HSCs induces high levels of DNA damage and activates a *p21*-dependent cell-cycle arrest in AML CSCs, which allows them to repair excessive DNA damage and to escape apoptosis, thereby maintaining their leukemic self-renewal capacity (Viale et al., 2009). While it may seem paradoxical that a leukemia-initiating oncogene promotes cell-cycle arrest instead of proliferation, the hijacking of such a protective mechanism provides a strong selective advantage to the CSCs. In the absence of *p21*, AML CSCs were more sensitive to replicative and therapeutic stress, and *p21* null HSCs expressing PML/RAR or AML1/ETO were unable to transplant the disease into recipient mice, indicating a failure to maintain CSC activity (Viale et al., 2009).

CSCs in Breast Cancer

The first evidence that solid tumors also contained cells with CSC properties came with the demonstration that in human breast cancer, $CD44^{+}CD24^{-/lo}$ cells are more clonogenic and, when transplanted in immunocompromized mice, are able to generate tumors that recapitulate the parental disease (Al-Hajj et al., 2003). Transcriptional profiling of murine mammary gland CSCs revealed increased expression of many DDR and DNA repair associated genes (Zhang et al., 2008), suggesting that mammary gland CSCs might be more resistant to chemo- and/or radiotherapy. Comparison of tumor biopsies before and after neoadjuvant chemotherapy showed an increase in the proportion of mammary gland CSCs with mammosphere-forming capacity following chemotherapy, hence confirming that mammary gland CSCs are more resistant to chemotherapy (Li et al., 2008; Shafee et al., 2008). Like normal MaSCs, mammary gland CSCs harbor lower levels of ROS compared to the rest of the tumor cells, due to increased levels of genes regulating free radical scavenging systems, such as those of the glutathione metabolism. Mammary gland CSCs from human xenografts (Phillips et al., 2006) or MMTV-Wnt1 tumor-bearing mice (Diehn et al., 2009) exhibited higher survival upon IR treatment. Consistent with the fact that ROS levels control IR-induced DNA damage and apoptosis in CSCs, inhibition of glutathione metabolism decreased the clonogenic potential and sensitized mammary gland CSCs to IR (Diehn et al., 2009). Furthermore, *p53*-deficient mammary gland CSCs show accelerated DNA repair activity as well as high Akt and Wnt signaling activity, which promotes CSC survival following IR treatment (Zhang et al., 2010). Interestingly, administration of an Akt inhibitor inhibits β-catenin signaling and sensitizes mammary gland CSCs to radiotherapy.

Understanding the role of DNA repair genes in the pathogenesis of breast cancer has been exploited for the development of novel anticancer strategies. Tumors derived from *Brca1*-deficient cells are extremely sensitive to the inhibition of PARP, which plays an important role in the repair of single-strand breaks by the BER pathway. In the absence of *Brca1* and HR-mediated DNA repair, persistent single-strand breaks need to be repaired by the BER pathways, and as a consequence, inhibition of PARP blocks this alternative pathway of DNA repair, inducing cell death preferentially in cancer cells. A PARP inhibitor prolonged disease-free survival when administered alone or in combination with chemotherapeutic drugs in a mouse model of *brca1*-deficient mammary gland tumors (Rottenberg et al., 2008) and also exhibits clinical efficacy in human breast cancers (Fong et al., 2009).

CSCs in Glioblastoma

Glioblastoma multiform (GBM) represents the most aggressive type of brain tumor. The standard treatment combines surgery and radiotherapy, but still, most patients relapse after therapy, with a median survival of less than 12 months (Prados and Levin, 2000). CSCs from human glioblastoma have been isolated based on the expression of prominin (CD133) (Singh et al., 2004). Irradiation of human GBM xenografts led to increased proportions of $CD133^{+}$ cells, indicating that CSCs may be responsible for tumor relapse after radiotherapy (Bao et al., 2006). CSCs from GBM are more resistant to IR-induced cell death compared to non-CSCs and show more robust activation of DNA-damage checkpoint proteins, including ATM, Chk1, and Chk2, as well as more efficient DNA repair activity. Importantly, treatment with inhibitors of Chk1 and Chk2 kinases sensitizes CSCs to IR-induced cell death, suggesting that inhibition of DNA-damage checkpoint in CSCs may improve the efficiency of radiotherapy in GBM (Bao et al., 2006). However, this increase in DNA repair activity was not observed in all glioma-derived cell lines (Ropolo et al., 2009), and loss of Chk2 instead potentiates GBM radioresistance in mice (Squatrito et al., 2010), indicating that this characteristic may be related to certain glioblastoma subtypes. Moreover, glioma stem cell-like cells have been shown to exhibit elevated levels of the antiapoptotic protein Mcl1 that contributes to their radioresistance (Tagscherer et al., 2008). Temozolomide, the most commonly used chemotherapy in the treatment of GBM that induces cell death by triggering the methylation of guanine at position 6, which can be

removed by the methylguanine DNA methyltransferase (MGMT), induced CSC depletion in MGMT-negative, but not in MGMT-positive, GBM (Beier et al., 2008).

Future Directions

The study of DDR in different types of tissue-specific SCs has clearly highlighted the existence of common mechanisms acting in certain adult SC populations to limit the amount of DNA damage, to restrain them from undergoing massive apoptosis and being exhausted following DNA damage, and to preserve overall tissue function. These protective mechanisms may have a cost for these tissue-specific SC populations, such as blood HSCs and hair follicle BSCs, as they preserve immediate survival at the expense of long-term maintenance of genomic integrity, which may lead to aging, tissue atrophy, and/or cancer development. Further studies are required to fully understand and ultimately prevent the long-term deleterious consequences of these protective mechanisms. In contrast, some tissue-specific SCs, such as intestinal SCs, are not well protected and undergo massive death after DNA damage. More studies are needed to better understand why some SCs prefer to commit suicide after DNA damage while others decide to survive, as well as to understand how altruistic suicide might provide a selective advantage to overall tissue function and what molecular mechanisms dictate these very different outcomes.

Most of the studies on DDR in tissue-specific SCs have been performed in adult animals during normal, or homeostasic, conditions. Since the activity and relative quiescence of SCs varies considerably during organogenesis, adult homeostasis, and tissue repair following injuries, the consequence of DNA damage might be very different in SCs at different ontogenic stages or levels of activity, as it has now been shown for fetal and adult HSCs. During organogenesis and tissue regeneration, SCs divide more frequently, whereas during homeostasis, SCs are more quiescent. Since different mechanisms of DNA repair are used depending on the cell-cycle stage of the damaged cells, are HR and NHEJ repair pathways differentially important to preserve SC fitness depending on their activation state? Are DNA repair-associated genes differentially activated during morphogenesis, homeostasis, and regeneration? Do mice with defective NHEJ or HR repair genes present different phenotypes when these genes are ablated during embryonic development compared to adult life? Future investigations are needed to fully comprehend the role of these different DNA repair mechanisms in SC biology.

In addition to the conserved set of genes that act in DDR and DNA repair pathways, some miRNAs have recently been shown to be induced by p53 in response to DNA damage and play an important role in DDR outcomes of survival versus apoptosis by interacting with key tumor-suppression networks (He et al., 2007). Irradiation of cultured cells uncovered the involvement of miR-34a in promoting apoptosis (Chang et al., 2007) and of miR-192 and miR-215 in cell-cycle arrest induction (Georges et al., 2008). Moreover, miR-34a is lost in several cancer cell lines (Chang et al., 2007). Future studies will determine whether DNA damage and repair-associated miRNAs are differentially expressed in tissue-specific SCs compared to their differentiated progeny and whether these miRNAs modulate the DDR in different types of tissue-specific and cancer SCs. Another important question is whether CSCs from different types of cancer also exhibit a survival advantage following chemo- and radiotherapy. If so, is this resistance related to enhanced DNA repair mechanisms or higher expression of antiapoptotic factors? Do CSCs retain the DNA repair properties of the SCs of their tissue of origin, or do they acquire functionally similar characteristics during cancer progression through a selective pressure? Do DDR abnormalities in CSCs versus bulk cancer cells account for the vast genomic instability present within the bulk of the tumors? Progresses in next generation whole genome sequencing and further studies of defined CSC populations will be needed to assess how defects in their DDR contribute to cancer evolution and associated genomic or base-pair level changes.

Addressing these open questions will have profound implications for our understanding of how tissue-specific SCs respond to DNA damage and maintain the integrity of their genome, how deregulation of these mechanisms leads to cancer and aging, how CSCs respond to chemo- and radiotherapy, and how these characteristics may be exploited to increase the efficacy of current anticancer treatments.

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