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Resilient and resourceful: genome maintenance strategies in hematopoietic stem cells

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Abstract

Blood homeostasis is maintained by a rare population of quiescent hematopoietic stem cells (HSC), which self-renew and differentiate to give rise to all lineages of mature blood cells. In contrast to most other blood cells, HSCs are preserved throughout life and maintenance of their genomic integrity is therefore paramount to ensure normal blood production and prevent leukemic transformation. HSCs are also one of the few blood cells that truly age and exhibit severe functional decline in old organisms, resulting in impaired blood homeostasis and increased risk for hematological malignancies. In this review, we present the strategies used by HSCs to cope with the many genotoxic insults that they commonly encounter. We briefly describe the DNA-damaging insults that can affect HSC function, and the mechanisms that are employed by HSCs to prevent, survive, and repair DNA lesions. We also discuss an apparent paradox in HSC biology, in which the genome maintenance strategies used by HSCs to protect their function in fact render them vulnerable to the acquisition of damaging genetic aberrations.

Introduction

DNA damage is a major driver of genomic instability. If left unrepaired or mis-repaired, DNA damage leads to mutations, which can result in loss of function or oncogenic transformation¹. To prevent this scenario, cells can sense DNA damage and activate a series of cellular mechanisms collectively referred to as the DNA damage response (DDR)². DDR can either promote cell survival through cell cycle arrest and DNA repair, or eliminate damaged cells through senescence or apoptosis³.

DNA is constantly damaged through a variety of intrinsic and extrinsic sources, and it has been estimated that up to 10⁵ DNA lesions occur daily in the genome of a cell⁴. Intrinsic sources such as reactive oxygen species (ROS) generated by mitochondrial respiration can cause oxidation of the nucleotide pool and DNA breaks, while spontaneous hydrolysis of DNA can lead to the formation of abasic sites and deamination. In addition, DNA replication or mitosis can give rise to replication errors, telomere attrition or chromosome missegregation. Extrinsic sources such as irradiation, UV or genotoxic agents can cause base

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modifications and single- or double-stranded DNA breaks (SSB/DSB). To repair these lesions, a cell can employ a broad array of DNA repair mechanisms, each designed to fix specific types of DNA lesions and to be used in particular phases of the cell cycle⁵.

DNA lesions including oxidative damage, UV-induced pyrimidine dimers, or DNA replication errors can be resolved at all stage of the cell cycle by base excision repair (BER), nucleotide excision repair (NER) or mismatch repair (MMR), respectively⁵. More complex DSBs are repaired by classical or alternative nonhomologous end-joining (NHEJ) throughout the cell cycle and by homologous recombination (HR) during the S/G₂ phases in cycling cells⁶. However, whether NHEJ and HR coordinate or compete for DSB repair during S/G₂ phases remains to be clarified. Importantly, the HR pathway is considered error-free as it uses the identical sister chromatid as a template for repair, which is only available in cycling cells. In contrast, NHEJ, especially for the alternative NHEJ pathway, is a more error-prone form of DSB repair as both ends of the broken DNA are directly ligated after processing. This can result in deletions, small insertions and even translocations if DSBs from different parts of the genome are aberrantly joined⁷. Therefore, both the type of DNA lesions and the choice of DNA repair pathway determine the fidelity of repair and the genomic stability of a cell.

Upon DNA damage, the DDR coordinates cell cycle progression, repair mechanisms, or the elimination of damaged cells. Key sensors of DNA damages are the Ataxia Telangiectasia Mutated (Atm) and Atm-and-Rad3-related (Atr) kinases. Atm primarily responds to DSBs through its downstream targets Chk2 and various DNA repair proteins⁸. Atr is mainly activated upon UV induced or replication-associated DNA damage through Chk1 phosphorylation, although there is considerable crosstalk between the two pathways⁹. Downstream of these DDR kinases, the induction of the tumor suppressors p16^{INK4A} and p19^{ARF} further propagate the DDR signal through modulating the retinoblastoma (Rb) and p53 pathways, respectively¹⁰. In this context, the p53 transcription factor might be the potent DDR mediator as it is able to orchestrate by itself apoptosis, senescence and cell cycle progression after DNA damage^{11,12}.

DNA damage is particularly dangerous when it occurs in stem cells. Stem cells are essential for the formation, maintenance and regeneration of most tissues in adult organisms due to their unique ability to self-renew and produce all types of differentiated mature cells^{13,14}. Preservation of stem cells genomic and functional integrity is therefore essential for proper tissue function. DNA damage can result in apoptosis leading to stem cell attrition and eventually tissue failure, or, upon misrepair, in accumulation of mutations eventually contributing to transformation and cancer development¹. Thus, the response of stem cells to DNA damage must be finely balanced to prevent these deleterious outcomes and maintain tissue homeostasis.

Blood-forming hematopoietic stem cells (HSC) are one of the best-characterized stem cell population¹⁵, with a wealth of information currently available on the molecular mechanisms controlling their functional properties including self-renewal activity, cell cycle regulation and differentiation potential^{16,17}. In an adult organism, HSCs reside in the bone marrow (BM) cavity in specialized “niches” that maintain them mainly inactive in the quiescent phase of the cell cycle. However, in response to the need of the organism, HSCs can undergo massive proliferation expansion and produce all types of mature cells needed for blood regeneration. While HSCs efficiently give rise to all blood cells in young individuals, they often fail with age, resulting in impaired blood production and the development of a broad spectrum of age-related blood diseases¹⁸. This functional decline of old HSCs has, among others, been attributed to the accumulation of DNA damage as based on increased levels of H2AX foci^{19,20}, a well-known indicator of DSBs. However, it remains

controversial whether H2AX is always a marker of acute or accumulated DNA damage in the absence of exogenous insults. Independently of the age of the stem cell compartment, DNA damage can also occur as a consequence of chemotherapy using DNA damaging agents and lead to the development of either acute myelosuppression and BM failure (BMF) syndromes, or therapy-related acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS)²¹. In addition, many inherited human diseases caused by mutations in DNA repair genes are manifested by hematological defects, BMF syndromes and an increased risk for leukemia^{22,23}. Therefore, determining how HSCs respond to DNA damage and maintain their genomic integrity is crucial for understanding the fundamental mechanisms of aging and leukemic transformation in the blood system.

Here, we review how DNA damage is generated in HSCs and how HSCs cope with insults to their genome. We mainly focus on recent insights gained from mouse studies on HSC genome maintenance strategies and only touch upon some of the main DNA repair mechanisms acting in HSCs and how their defects contribute to congenital and acquired blood diseases in humans, since these aspects have been covered in detail in recent reviews^{23–25}. We describe how the unique molecular wiring of HSCs both safeguards them against the occurrence of DNA damage and ensures their survival but, paradoxically, also makes them susceptible to acquiring mutations and genomic instability.

Mechanisms that prevent DNA damage in HSCs

Given the lifelong potential for genomic lesions to hamper HSC function, one solution selected by evolution has been to develop an array of protective mechanisms that minimize their occurrence in the first place. DNA lesions can, among others, originate from replicative, oxidative or environmental stress. Since these genotoxic stresses are interconnected, it is logical that the strategies to minimize them are often overlapping. Figure 1 summarizes the main protective strategies employed by HSCs to ensure their function and minimize genomic instability.

Minimizing replication stress through quiescence

The main protective strategy against replication stress is to restrict HSCs to a quiescent or dormant (G₀) cell cycle state that prevents DNA damage associated with DNA replication and mitosis^{17,26,27}. Quiescence is unique to adult HSCs, as fetal or postnatal HSCs are predominately found in an active cell cycle state^{15,17}. Within the adult pool, HSCs with the largest self-renewal capacity also divide the least – once every 145 days – roughly 5 times during the lifespan of a mouse²⁶. However, most mouse HSCs divide once every 30 days to ensure homeostatic blood production¹⁷. In parallel, simulation studies in humans propose that HSCs divide every 40 weeks indicating that the numbers of HSC replications per lifetime are comparable between mouse and human²⁸. Actively cycling HSCs exhibit reduced self-renewal capacity, and many studies have correlated the loss of quiescence and increased proliferation activity to functional decline of both murine and human HSCs^{27,29}. The importance of quiescence for HSC function is further demonstrated by the unique molecular wiring of their cell cycle machinery and the predominance of p53 and the cyclin D1/p57 axis in enforcing quiescence^{17,26}. The switch between quiescence and cell cycle progression appears to be regulated in a more stringent and non-redundant fashion in HSCs compared to other cell types, since disruption of cell cycle proteins often results in HSC-specific phenotypes. One example is *cyclin A2*, which is essential for HSC proliferation but dispensable for fibroblast proliferation due to redundancy with *cyclin E*³⁰. Another example is *p57* inactivation, which has no effect on hematopoietic progenitors but leads to loss of HSC quiescence, decrease in HSC number and function, and increase in p53-mediated apoptosis^{31,32}. Overall, quiescence is essential for the maintenance of HSCs in adult

organisms, and acts as the main protective mechanism against replication-associated DNA damage.

Minimizing oxidative stress through ROS regulation

Normally, HSCs have low ROS levels which serve as important signaling molecules for differentiation³³, whereas myeloid progenitors have 100-fold higher ROS levels³⁴. Excessive ROS are detrimental for HSC function, and one of the first studies demonstrating a direct link between high ROS and loss of HSC function was actually conducted in mice deficient for the DDR gene *Atm*. *Atm*-deficient mice display a contraction of the HSC pool with decreased self-renewal capacity and loss of quiescence, which ultimately leads to HSC exhaustion associated with the development of age-associated BMF syndrome and increased p16^{INK4A}/p19^{ARF} expression^{35,36}. Loss of HSC function is caused by elevated ROS and consequent activation of the p38 MAPK pathway, as reducing ROS through treatment with an antioxidant, N-acetylcysteine (NAC) or p38 inhibition rescue *Atm*-deficient HSC function^{35,36}. Furthermore, lymphoma development in *Atm*-deficient mice could be delayed by antioxidant treatment, demonstrating that excessive ROS directly lead to genomic instability and transformation in this mouse model³⁷. The importance of maintaining low ROS appears as a general requirement for HSC maintenance. For example, HSC deficient in all 3 FoxO transcription factors (*FoxO1*, *FoxO3*, and *FoxO4*) or *FoxO3a* alone lose quiescence, have increased apoptosis and reduced self-renewal capacity^{34,38}. FoxO transcription factors control ROS by regulating genes involved in ROS detoxification and cell cycle progression^{34,39}. The loss of HSC function in the *FoxO1/3/4*-deficient mouse model is again a consequence of high ROS levels, as reducing ROS through antioxidant treatment restored HSC quiescence and function^{34–36}. Functional studies in both humans and mice cells have also shown that chemical induction of oxidative stress or serial transplantation result in elevated ROS, increased HSC proliferation and HSC functional exhaustion, which can be inhibited by antioxidant treatment or p38 inhibition^{29,36}. In these contexts, high ROS levels are also directly correlated to increased DNA damage, as assayed by γH2AX foci and expression of proteins involved in the DDR pathway²⁹. Taken together, these results demonstrate that oxidative stress can cause DNA damage and contribute to loss of quiescence and functional impairment in HSCs.

While it is tempting to speculate that ROS-induced DNA damage is the main driver of reduced HSC function in *Atm*- or *FoxO*-deficient mouse models, its involvement has not been formally demonstrated. Recent studies suggest an additional layer of complexity since *Atm* also acts as a redox sensor independently of its role in the canonical DDR pathway, and activates cell-cycle checkpoints in response to oxidative stress⁴⁰ and deregulated mitochondrial homeostasis⁴¹. Therefore, low or absent *Atm* could impair HSC function by both failing to induce a correct checkpoint response after high ROS levels and impairing the repair of ROS-mediated DNA damage. Interestingly, deletion of all three *FoxO* transcription factors or *FoxO3* alone leads to decreased expression of *Atm*^{34,42} hence linking together at least two of the important regulatory pathways controlling oxidative stress. In turn, *Atm* could regulate the response to oxidative stress and low levels of DNA damage in HSCs in part by phosphorylating Bid, a BH3-only pro-apoptotic BCL2 family member⁴³. In the absence of *Atm*, HSC have increased apoptosis following irradiation, loss of quiescence, decreased self-renewal, and increased ROS due to Bid accumulation in mitochondria⁴³. These studies illustrate the central role that *Atm* plays in dampening the mutagenic consequences of oxidative stress in HSCs through regulation of DNA repair, cell cycle and apoptosis. Collectively, they show that keeping ROS levels in check and minimizing oxidative stress is critical for HSC genomic stability.

Minimizing genomic instability through checkpoint activation

Checkpoint activation is a potent strategy to minimize genomic instability². Mice with deletion of the Polycomb repressor *Bmi1* display reduced HSC self-renewal capacity, high ROS, and shortened life span⁴⁴. In addition, disruption of *Bmi1* leads to activation of the DDR pathway demonstrated by *Chk2* expression and increased expression of *p16^{Ink4a}* and *p19^{Arf}*⁴⁴. Interestingly, lowering ROS or inactivation of the DDR through *Chk2* disruption increase the lifespan of *Bmi1*-deficient mice but does not restore HSC self-renewal⁴⁴. This demonstrates that HSC function is hampered in the absence of Bmi-1, independently of high ROS. This is likely due to the continued activation of *p16^{Ink4a}* and *p19^{Arf}* since their expression levels are not normalized after antioxidant treatment or *Chk2* disruption⁴⁴. HSCs lacking *Pten*, a phosphatase that inactivates PI3-K signaling and negatively regulates the mTOR pathway, also display increased cell cycle activity and impaired self-renewal capacity, with *Pten*-deficient mice succumbing to leukemia^{45,46}. However, loss of quiescence in *Pten*-deficient HSC is not accompanied by increased ROS or DNA damage⁴⁷. Rather, *Pten* deletion results in HSC depletion through expression of the tumor suppressor *p16^{Ink4a}* and *p53*, and accelerated leukemogenesis is observed in mice with combined deletion of *Pten* with *p16^{Ink4a}/p19^{Arf}*, *p19^{Arf}*, or *p53*⁴⁷. The mechanism by which activation of these tumor suppressors perturb HSC function and accelerate *Pten*-mediated tumorigenesis is unclear, but neither increased senescence nor increased apoptosis are observed. Rather it is speculated that they promote HSCs exhaustion and induce differentiation⁴⁷. Several recent reports also support the idea that checkpoint activation stimulates differentiation and limits HSC self-renewal upon DNA damage^{48,49}. Following DDR activation due to γ -irradiation or telomere shortening, HSCs up-regulate the transcription factor *Batf* in a STAT3/G-CSF dependent manner, which stimulates HSC differentiation at the expense of HSC maintenance⁴⁸. As expected, while down-regulation of *Batf* expression improves HSC self-renewal, it also results in accumulation of DNA damage in HSCs. Moreover, disruption of both the *p16^{Ink4a}/p19^{Arf}* and *p53* loci lead to the acquisition of long-term reconstituting ability in normally non self-renewing multipotent blood progenitors, which suggests that these tumor suppressor genes are directly involved in limiting self-renewal activity in progenitor cells⁴⁹. Collectively, these results indicate that checkpoint activation and induction of differentiation following DNA damage are mechanisms used to prevent the accumulation of damaged HSCs. However, such strategies and their associated consequences such as HSC exhaustion, may lead to impaired blood homeostasis and have detrimental effects with age.

Minimizing oxidative stress through metabolic regulations

HSCs reside in endosteal niches close to the bone surface and in perivascular niches adjacent to sinusoidal blood vessels, which are formed by many different BM stromal populations⁵⁰. These specialized BM niches provide cytokines that not only control HSC quiescence (*i.e.*, TGF β , CXCL12, SCF, etc.) and differentiation activity (*i.e.*, IL-7, M-CSF, etc.), but also play an important role in maintaining HSC genomic stability. In particular thrombopoietin (TPO), an important regulator of platelet production, stimulates DNA repair by increasing DNA-PK-dependent NHEJ efficiency in damaged HSCs and thus limits mutagenesis in response to DNA damage⁵¹. In addition, the BM niche provides a hypoxic environment that maintains low ROS in HSCs^{52–54}, which is essential for HSC function since cells with the lowest ROS levels have the highest reconstitution activity⁵⁵. Within these hypoxic BM niches, HSCs are limited to anaerobic glycolysis for ATP production instead of mitochondrial oxidative phosphorylation, through regulations enforced by the *Hif-1* transcription factor^{56,57}. Mice lacking *Hif-1* display reduced HSC self-renewal capacity, loss of quiescence and increased ROS⁵⁴. Recent metabolomic studies and genetic disruption of the *Pdk2/4* glycolytic enzymes confirm that active suppression of mitochondrial respiration is essential to maintain HSC quiescence and self-renewal

activity⁵⁸. These studies demonstrate that quiescent HSCs residing in a hypoxic environment rely on anaerobic glycolysis as an energy source, which in turn limits the generation of oxidative stress. Collectively, they suggest that BM niches protect HSCs from genomic instability by both preventing the generation of DNA damage through metabolic regulation, and promoting DNA repair through paracrine factors such as TPO.

However, proliferating HSCs have to increase their energy production and engage mitochondrial respiration since inhibition of this process results in impaired differentiation⁵⁹. For example, mice lacking *Ptpmt-1*, a mitochondrial phosphatase necessary for oxidative phosphorylation, show an expanded quiescent HSC compartment due to an inability to differentiate and produce mature blood cells, hence leading to impaired HSC self-renewal and BMF syndrome^{54,59}. In accordance, HSCs have a relatively high mitochondria content, with mitochondria that are however less active than in cycling progenitors⁶⁰. This supports the idea that quiescent HSCs are primed for the large metabolic activity required for regenerating the blood system. Interestingly, the accumulation of mutations in mitochondrial DNA is selectively more toxic to lymphoid progenitors than to HSCs, indicating that HSC's reduced dependence on mitochondrial energy may also mitigate the effects of mitochondrial genomic instability⁶⁰. These data argue for a dynamic metabolic wiring in HSCs, with a constitutive low energy state enforced by the hypoxic BM niches that limits exposure to oxidative damage by preventing the engagement of mitochondrial respiration and ROS production, and maintain HSC quiescence. However, HSCs can rapidly transition into a more energy producing state necessary for the transient proliferation associated with self-renewal and differentiation activity, which then increases ROS levels and promotes exposure to oxidative damage⁶¹.

Mitochondrial membrane integrity and lipid metabolism are also instrumental for HSC function^{62–64}. In response to energy stress, *Lkb1*, a kinase known to activate several AMP-activated protein kinases (AMPK), inhibits the canonical mTOR pathway and impedes protein translation and cell growth. Deletion of *Lkb1* results in loss of quiescence and increased proliferation accompanied by HSC exhaustion^{62–64}. Interestingly, only *Lkb1*-deficient HSCs but not their progeny have decreased mitochondrial potential and increased mitochondrial mass, suggesting an HSC-specific metabolic rewiring in the absence of *Lkb1*. This is not mediated by increased ROS or through the mTOR pathway since *Lkb1*-deficient HSC phenotypes are not rescued by NAC or rapamycin treatment. Rather, *Lkb1*-deficient HSCs display increased DNA damage as assayed by increased H2AX foci⁶³ and aneuploidy⁶⁴. Disruption of mitochondrial fatty acid oxidation (FAO) also leads to loss of quiescence and diminished HSCs self-renewal due to perturbation in the rate of asymmetric cell division⁶⁵. Deregulation of FAO metabolism could provide a molecular basis for the phenotype of *Lkb1*-deficient HSCs, which show down-regulation of important components of the FAO pathway such as *Pgc1a*⁶². Together, these studies indicate that HSCs have a tightly regulated and unique metabolic wiring that protects against genotoxic stress and is essential for HSC self-renewal perhaps in part due to governing the rate between asymmetric and symmetric divisions.

Minimizing environmental stress through detoxification

HSCs are also equipped with specific detoxification mechanisms. In comparison to progenitors, HSC express high levels of the ATP-binding cassette (ABC) transporters that facilitate the efflux of genotoxins⁶⁶. Mice that lack the main ABC transporter *Bcrp1* have normal HSC number and function. However, *Bcrp1*-deficient HSCs are more sensitive to the toxic effects of genotoxic agents in competitive transplantation assays, which demonstrates that ABC transporters are also important in protecting HSC from genomic damage⁶⁷. Recent work shows that detoxification of acetaldehydes is also critical for HSC genomic integrity^{68,69}. Aldehydes are reactive metabolites that can induce DNA-DNA and DNA-

protein crosslinks, which lead to DNA breaks. In contrast to progenitors, HSCs depend on aldehyde dehydrogenase 2 (*Aldh2*) for protection against acetaldehyde toxicity. When these reactive molecules persist, DNA damage occurs selectively in the HSC compartment and this necessitates DNA repair through the Fanconi anemia (FA) DNA repair pathway²⁵. Mutations in one of the 16 FA or FA-like genes lead to severe hematological abnormalities including BMF syndrome and blood malignancies in almost all FA patients by age 40^{25,70,71}. Recent work shows that the BMF syndrome in both FA patients and FA pathway deficient mice is due to an exacerbate p53/p21-mediated DDR that impairs HSC and progenitor cell proliferation⁷². A severe reduction of the HSC pool is also observed in FA pathway and acetaldehyde detoxification deficient mice, suggesting that acetaldehyde-mediated genotoxicity could directly contribute to HSC exhaustion and BMF syndrome^{68,69}. Thus detoxification pathways that reduce exposure to harmful metabolic or other toxic compounds are critical to maintain HSC function and genomic stability.

Mechanisms that ensure survival in HSCs

In addition to DNA damage evasion, another strategy to mitigate genomic insults and maintain lifelong blood production is an idiosyncratic molecular network poising HSCs for survival⁷³. Although the BM is among the most radiosensitive organs, HSCs are one of the blood cells most resistant cells to IR exposure in adult mice^{74,75}. This enhanced survival is due in large part to the high expression of pro-survival members of the *Bcl2* gene family in HSCs⁷⁶. In accordance, forced overexpression of *Bcl2* in the hematopoietic compartment protects mice from radiation-induced hematopoietic failure⁷⁷. In contrast, the opposite response is observed during fetal development in humans as cord blood HSCs are more sensitive to IR exposure than progenitors, and overexpression of pro-survival Bcl2 proteins or down-regulation of p53 can rescue this hyper-sensitivity⁷⁸. These opposing results suggest that the pro-survival Bcl-2 machinery is perhaps not yet fully wired in fetal HSCs in contrast to adult HSCs where this pro-survival network might already be at maximal strength⁷⁶. This difference between fetal and adult HSCs is perhaps not surprising given that fetal HSCs are an expanding, highly proliferative population that are building the blood system, whereas adult HSCs are a finite quiescent population that are geared towards maintaining blood production²³. In addition, it could also point to differences between mice and humans with respect to the wiring of their DDR and the activity or expression of some repair enzymes⁷⁹.

Strategies ensuring survival at the population level also contribute to HSC resilience. Transplantation experiments with BM cells isolated from either untreated or IR-exposed mice show that HSCs with low p53 levels or mutant p53 have a strong competitive advantage, which is independent from the canonical p53-mediated DDR and results from frequency-dependent selection^{80,81}. These results indicate that HSCs with mutant or low level p53 outcompete HSCs that express high levels of p53 in a non-cell autonomous manner. Moreover, they demonstrate the existence of a selection mechanism that upon DNA damage favors the expansion HSCs with lower checkpoint signaling and p53 inactivation. While this strategy allows HSC maintenance at the population level and therefore protects blood production, it also likely contributes to the accumulation of p53-deficient clones and leukemia development^{82,83}.

Another HSC survival strategy is autophagy, a process by which damaged cellular components are sequestered within autophagosomes and degraded, which serves as a major stress response mechanism to protect from metabolic starvation⁸⁴. Autophagy is essential in fetal HSCs as shown in mice lacking the essential autophagy gene *Atg7*⁸⁵. *Atg7*-deficient HSCs are quickly exhausted after birth and display an accumulation of mitochondria, increased ROS, loss of quiescence and DNA damage. Autophagy is also essential to protect

adult HSCs from starvation⁸⁶. This is unique to the HSC compartment and depended on FoxO3A, which maintains a pro-autophagy gene expression program, that poises HSCs for rapid induction of a protective autophagy response. In addition, HSCs isolated from aged mice are constitutively metabolically stressed and rely on high basal levels of autophagy for survival⁸⁶. Overall, HSC's ability to engage autophagy upon metabolic stress safeguards HSC survival but ongoing autophagy in old HSCs could help maintain damaged HSCs and thus contribute to age-associated blood defects.

Mechanisms that ensure DNA repair in HSCs

The ability to repair DNA damage and prevent genomic instability is crucial for HSCs maintenance²³. Mice deficient in telomere maintenance components, such as naturally mutated in Dyskeratosis Congenita (DC) syndrome patients, or DNA repair pathway components such as NER or NHEJ all show HSC functional exhaustion due to their depletion by apoptosis or senescence^{19,87–90}. In addition telomere dysfunction also induces alterations in the microenvironment limiting HSC engraftment potential⁹¹. These findings demonstrate the need for ongoing DNA repair to preserve HSC function throughout life. However, DNA repair also contributes to genomic instability in HSCs. As HSCs are predominantly quiescent they are restricted to using NHEJ to repair IR-induced DSBs⁷⁶. As NHEJ is an error-prone DNA repair pathway, quiescent HSCs are therefore prone to acquire mutations and chromosomal instability following DNA repair. Interestingly, HSCs forced to proliferate acquire fewer mutations after IR-induced DSBs, as they use the high fidelity HR repair mechanism⁷⁶. These results demonstrate that while quiescence protects HSCs by limiting genomic insults, it also renders them vulnerable to mutations following DNA damage. This paradoxical finding illustrates one recurrent features of the strategies designed to limit DNA damage and favor survival in HSCs, which is that they often involve a trade off between functional maintenance and genomic integrity (Figure 2). Recent whole genome sequencing studies of normal human BM cells as well as primary and relapsed MDS/AML patient samples has given insight into the mutational landscape associated with normal aging and disease progression, and provided direct evidence that HSC acquire mutations throughout life^{92–94}. While transformed MDS/AML cells contain known driver oncogenic mutations, healthy cells also accumulate mutations with age, which are mostly non-pathogenic mutations⁹⁴. In addition, increased genomic instability probably due to chemotherapy-induced DNA damage is observed in primary and relapsed AML sample pairs in which the treatment fails to eradicate the malignant clones⁹². These human sequencing studies demonstrate that HSCs acquire mutations with age and upon treatment with genotoxic drugs, which might be a direct consequence of quiescence-restricted usage of error-prone NHEJ repair mechanisms in HSCs. Taken together, these results illustrate how survival mechanisms designed to ensure the best HSC regenerative capacities can over time contribute to the accumulation of DNA damage and disease development.

Concluding remarks

This review illustrates the reliance of HSCs on pro-survival mechanisms to preserve their function and ensure maintenance of blood homeostasis when confronted with DNA damage, as well as HSC's resourcefulness in preventing exposure to endogenous genotoxins and limiting acquisition of DNA damage. However, these genome maintenance strategies are a double-edged sword as they can also drive genomic instability and oncogenic transformation when DNA damage is repaired through error-prone mechanisms in quiescent HSCs (Figure 2). In addition, over-activation of the DDR machinery can have severe deleterious consequences for blood homeostasis as evidenced by the BMF and anemia diseases resulting from hyperactivated p53 pathway^{72,95,96}. Further study of HSC genome maintenance strategies will therefore lead to a greater understanding of the pathogenesis of human blood

disorders, which arise as a consequence of specific types of chemotherapy damages occurring in HSCs, or are inherited due to mutations in essential HSC protective mechanisms or DNA repair pathways. In particular, they could shed new lights on the etiology of many congenital BMF syndromes such as FA and DC, and lead to better therapeutic options.

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Figure 1. Mechanisms preventing genomic insults in HSCs

The sources of genomic insults are depicted in black and the mechanisms used by HSCs to minimize their effects are shown in red.

Figure 2. Sources and consequences of genomic insults in HSCs

The main mechanisms that can either induce or protect against DNA damage in HSCs are summarized. Their outcomes for blood production are depicted in green for beneficial effects, and red for detrimental effects.