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DNA repair is crucial for maintaining hematopoietic stem cell function

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Abstract

Richard Cornall and collaborators recently developed a mouse model of Ligase IV syndrome with growth retardation and immunodeficiency due to a defect in nonhomologous end-joining (NHEJ) of DNA double-strand breaks. They demonstrated age-dependent loss of hematopoietic stem cell function in these mice. Simultaneously, Irving Weissman and colleagues demonstrated a similar phenomenon in *Ku80*^{-/-} mice defective in NHEJ and telomere maintenance, *Xpd*^{TTD} mice defective in nucleotide excision repair, and late generation *mTr*^{-/-} missing telomerase activity. These studies strongly support the hypothesis that genomic stress causes aging by limiting the ability of stem cells to indefinitely maintain tissue homeostasis.

Keywords

aging; progeria; oxidative stress; endogenous damage

A topic of keen interest in the field of genome maintenance is whether distinct cell types have different capacities for DNA repair and therefore sensitivity to genotoxic stress [1,2]. Tissue-specific stem cells are of particular interest [3,4] since these cells are responsible for organ development, sustaining tissue homeostasis and tissue regeneration after injury [5]. Maintaining stem cells throughout the entire lifespan of an organism is essential for preserving both tissue function and the ability of that organ to respond to stress [6]. Aging itself is defined as the loss of homeostatic reserve [7]. Thus loss of tissue-specific stem cells is predicted to promote aging [8,9].

Tissue-specific stem cells are also implicated in carcinogenesis. Stem cells have several unique characteristics that make them a logical nidus for cancer. They are long-lived, increasing the odds that a single cell could acquire the multiple mutations necessary for transformation. Stem cells also have an unlimited capacity for cell division and an ability to spawn multiple cell types, characteristics shared with tumors [10]. Furthermore, the quiescent nature of stem cells renders them resistant to chemotherapy [11] and therefore a potential source of tumor recurrence. Thus, determining how stem cells respond to genotoxic stress is critical for understanding the fundamental mechanisms of aging and cancer.

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Two recent manuscripts provide great inroads into revealing how stem cells respond to DNA damage through the study of hematopoietic stem cells (HSCs) in DNA repair deficient mice [12,13]. Nijnik *et al.* examined HSC number and function in Lig4-deficient mice (*Lig4*^{Y288C}), defective in non-homologous end-joining (NHEJ) repair of DNA double-strand breaks (DSBs). Rossi *et al.* did similar experiments using three mouse models of accelerated aging: *Xpd*^{TTD} mice, defective in nucleotide excision repair of helix-distorting DNA lesions [14], *Ku80*^{-/-} mice defective in NHEJ of DSBs and telomere maintenance [15,16], and *mTR*^{-/-} mice missing telomerase activity [17]. In all cases, HSCs from these DNA repair-deficient mice were compromised prematurely relative to normal mice, demonstrating that three major genome protection mechanisms are required for maintenance of stem cells, at least in the hematopoietic system. The major implication is that stem cells are vulnerable to endogenous DNA damage and that failure to repair this damage limits stem cell function.

Mammalian hematopoiesis

Hematopoiesis in adult mammals occurs in the bone marrow (BM). Replacement of both myeloid (granulocytes, monocytes, platelets, red blood cells) and lymphoid (B, T and natural killer cells) lineages is dependent upon long-term reconstituting hematopoietic stem cells (LT-HSCs). LT-HSCs can be isolated from the BM based on their unique pattern of surface markers (lineage⁻, c-Kit⁺, Sca-1⁺, flk2/CD135⁻, CD34⁻). The definition of stem cells encompasses a functional component (Figure 1). All stem cells have the unique capacity to divide to produce a phenocopy of themselves (self-renewal) and a more differentiated cell, which in the BM is the short-term reconstituting HSC (ST-HSC; lineage⁻, c-Kit⁺, Sca-1⁺, flk2/CD135⁻, CD34⁺). ST-HSCs give rise to more committed multi-potent progenitor cells (MPP; lineage⁻, c-Kit⁺, Sca-1⁺, flk2/CD135⁺, CD34⁺), which in turn give rise to the common lymphoid progenitors (CLPs) that produce lymphoid lineages and the common myeloid progenitors (CMPs) that produce myeloid lineages. The extent of lineage commitment parallels proliferation rate, meaning that the pluripotent LT-HSCs are relatively quiescent compared to the more committed progenitors.

DNA repair is not required to maintain hematopoietic stem cell number

To quantify HSCs, both groups measured the percent of BM cells with LT-HSC-specific surface markers in normal and DNA repair deficient mice at various ages using flow cytometry (Table I). There is a marked decrease in BM cellularity and the MPP compartment as early as 7 weeks of age in Lig4-deficient mice relative to age-matched controls [12]. However, the LT-HSCs and ST-HSCs are conserved in mice as old as 6 months of age. Similarly, the fraction of LT-HSCs and ST-HSCs in *Xpd*^{TTD} and third generation *mTR*^{-/-} mice (with shortened telomeres) is conserved up to ~1 year of age and in *Ku80*^{-/-} mice for at least 30 weeks [13]. In fact, the frequency of LT-HSCs increased significantly with age in each of these progeroid mouse models, identical to what is observed in wild type mice [13]. However, like the Lig4-deficient mice, the fraction of progenitor cells is significantly reduced in all three progeroid mouse strains relative to controls, even in young adult mice [13]. This demonstrates that defects in multiple mechanisms required for genome stability (nucleotide excision repair, NHEJ and telomere maintenance) are not essential for establishing or maintaining the relatively quiescent LT-HSCs. However, maintenance of the more proliferative progenitor lineages is dependent on these DNA repair mechanisms.

DNA repair is required to maintain hematopoietic stem cell function

To measure the function of HSCs, LT-HSCs were isolated from DNA repair-deficient mice at various ages and used to competitively transplant lethally irradiated mice. Fifty LT-HSCs from a DNA repair-deficient mouse were combined with 2×10⁵ BM cells from a control animal to determine if the stem cells could reconstitute the BM of the irradiated host. Read-outs included

measuring the fraction of lymphocytes and myeloid cells in the peripheral blood of the irradiated host at various time points post-transplantation to determine the short- and long-term reconstituting capacity of the HSCs. Circulating cells derived from LT-HSCs are distinguished using a strain-specific marker on the surface of the white blood cells (CD45.2 vs. CD45.1 for cells derived from the competitor BM or irradiated host).

The fraction of myeloid and lymphoid cells derived from LT-HSCs isolated from progeroid *Xpd*^{TTD}, *mTR*^{-/-} and *Ku80*^{-/-} mice was significantly decreased compared to transplants from age-matched wild type mice [13]. Granulocytes turn-over rapidly. Thus measuring the fraction of granulocytes stemming from LT-HSCs transplanted into an irradiated host (granulocyte chimerism) offers a near real-time assessment of HSC function. The fraction of granulocytes derived from adult *Xpd*^{TTD}, *mTR*^{-/-}, *Ku80*^{-/-} and *Lig4*^{Y288C} mouse LT-HSCs was significantly lower than wild type controls and continued to decrease with time post-transplantation, demonstrating exhaustion of HSC function when genome maintenance is compromised [12,13]. Importantly, the dramatic difference in granulocyte chimerism afforded by transplantation of wild type or DNA repair deficient LT-HSCs is not observed if younger donors are used [13]. Thus while LT-HSC numbers are conserved with ageing in *Xpd*^{TTD}, *mTR*^{-/-}, *Ku80*^{-/-} and *Lig4*^{Y288C} mice with defective genome maintenance, HSC function is lost prematurely in a cell autonomous fashion. This suggests that loss of hematopoietic reserves is caused by the accumulation of DNA damage in HSCs.

Loss of stem cell function is due to impaired self-renewal

Accumulation of DNA damage in HSCs could impair stem cell function by preventing replication and cell division, inducing apoptosis, or causing differentiation such that the HSCs can no longer self-renew. Rossi *et al.* measured self-renewal in vivo by quantifying donor LT-HSCs in the BM of transplanted mice. In mice transplanted with stem cells from aged *Xpd*^{TTD}, *mTR*^{-/-} or *Ku80*^{-/-} mice, the number of LT-HSCs was reduced 5, 16 and 26-fold, respectively, compared to mice transplanted with stem cells from wild type mice [13]. This inability to self-renew appears to be partly due to decreased proliferation of LT-HSCs. Growth of LT-HSCs derived from the progeroid mouse strains in vitro is significantly decreased compared to HSCs from wild type mice. In vivo, proliferation of HSCs and progenitors, measured by BrdU incorporation, is 2-fold greater in *Lig4*^{Y288C} mice compared to age-matched controls [12]. Since BM cellularity and progenitor populations are significantly reduced in these mice, it implies that there must be a mechanism of cell loss. Indeed, LT-HSCs from DNA repair deficient mice are more prone to spontaneous apoptosis in vitro [13]. Thus loss of genome protection mechanisms leads to decreased HSC self-renewal either by preventing proliferation of the HSCs or promoting apoptosis of progeny cells.

Age-dependent accumulation of DNA damage causes loss of stem cell function

The best evidence that DNA damage is ultimately responsible for the loss of HSC function comes from the *Lig4*-deficient mice. That is because *Lig4* functions exclusively in NHEJ repair of DSBs [18,19]. Therefore, the hematopoietic phenotype of *Lig4*^{Y288C} mice must be a direct consequence of failure to repair spontaneous DSBs. In accordance, γ -H2AX foci, a marker of DSBs [20] and replication stress [21] are significantly increased in HSCs isolated from *Lig4*-deficient mice compared to control littermates [12]. Furthermore, γ -H2AX foci are increased in HSCs isolated from old wild type mice compared to young [13]. Thus there is an inverse correlation between DNA damage and HSC function.

This new data brings the number of mouse models with defective genome maintenance and reduced HSC function to ten (Table I). These include mice defective in six different

mechanisms of genome maintenance, including NHEJ, nucleotide excision repair, homologous recombination-mediated DSB repair, interstrand crosslink repair, mismatch repair and telomere maintenance. Defects in any one of these mechanisms causes premature exhaustion of the BM as demonstrated by decreased ability to reconstitute the BM of an irradiated host in a competitive transplant assay (Table I). This inability to reconstitute BM becomes more pronounced with increased age of the donor and at late time points following transplantation (long-term reconstitution is more severely affected than short-term reconstitution), implicating loss of LT-HSC function as causal.

HSCs from mice defective in homologous recombination-mediated DSB repair are the most severely affected. Rad50 and ATM-deficient mice have frank BM failure (acellular marrow) by 8 and 24 wks of age, respectively [22,23]. The BM of adult *Brca2* and *Lig4* mutant mice is hypocellular [12,24]. Mice with defects in nucleotide excision repair, NHEJ repair of DSBs, DNA interstrand crosslink repair, mismatch repair or telomerase maintain normal BM cellularity into adulthood, suggesting a milder defect in HSC function. Interestingly mice expressing a hyperactive mutant allele of p53 that causes premature aging, also have impaired HSC function [25].

Model of how accumulated DNA damage impairs hematopoiesis

There is now overwhelming evidence that DNA damage limits stress-induced hematopoiesis. It does so by diminishing the ability of HSCs to proliferate and self-renew. It is worth emphasizing that this was demonstrated in DNA repair-deficient mice that were not exposed to exogenous genotoxic stress. Thus spontaneous or endogenous DNA damage is responsible for the loss of HSC function. Damage accumulation is not replication-dependent: DSBs accumulate in *Lig4*^{Y288C} mouse embryonic fibroblasts cultured in stationary phase at physiological oxygen tension [12]. Therefore the genome of quiescent HSCs is susceptible to spontaneous DNA damage. In fact, more DNA damage is detected in HSCs than progenitor cells isolated from aged mice [13].

This could imply that progenitor cells are more prone to apoptosis or that they have a greater capacity for DNA repair relative to HSCs. In fact, there is reason to suspect both. Homologous recombination-mediated DSB repair requires a sister chromatid. Thus this repair pathway is restricted to S/G2 phases of the cell cycle [26] and limited to proliferating cells such as progenitors. Furthermore, quiescent stem cells have been demonstrated to be relatively resistant to apoptosis compared to progenitors [10,27].

A key difference between progenitor cells and stem cells is their proliferation rate. The more rapidly dividing progenitors are preferentially lost in the absence of DNA repair, while quiescent stem cells persist and accumulate damage. This suggests that exit from G0 or entry into S phase of the cell cycle may be the point at which HSCs respond to a damaged genome. What is not clear is if HSCs with damaged genomes fail to enter S phase (replicative senescence) or initiate replication but undergo apoptosis. Regardless, the net result is that DNA damage accumulates in quiescent cells, while causing attrition of proliferating cells (Figure 2). Thus the BM of aged organisms is hypocellular, enriched for LT-HSCs and has diminished reconstituting capacity.

Implications for human aging

DNA repair deficient mice that mimic human syndromes are extremely valuable not only for understanding rare genetic diseases but also for discovering the health impact of endogenous DNA damage. The demonstration of impaired HSC function in *Xpd*^{TTD}, *Ku80*^{-/-} and *Lig4*^{Y288C} mice leads to the prediction that individuals with increased oxidative stress (*e.g.*, Wilson's disease) and cancer survivors treated with genotoxins are at risk of premature aging

of the hematopoietic system. Similarly, the loss of HSC function in late generation *mTr*^{-/-} mice leads to the prediction that hematopoietic stress, for instance caused by chronic inflammation or infection, may accelerate aging of the hematopoietic system.

The discovery that age-dependent accumulation of DNA damage limits stem cell function also warrants consideration when using adult stem cells for transplantation. Evidence that donor age affects outcome in BM transplantation is scant. In mice, there is increased skewing of engraftment towards myeloid lineages [28] and decreased immune reconstitution [29] if the donor is aged, consistent with impaired HSC function. In humans, donor age is inversely proportional to five year survival after BM transplantation, but primarily due to increased risk of graft versus host disease [30]. However, telomere length is greater in recipients transplanted with cells isolated from umbilical cord blood rather than adult blood, consistent with greater regenerative capacity of cells isolated from a young donor [31].

Although DNA repair deficient mice have decreased HSC function, the mice themselves don't all become pancytopenic or symptomatic as a direct consequence of HSC exhaustion. So is the reduced HSC function observed in these model systems likely to impact the health or longevity of humans with normal DNA repair? There are several reasons to consider the possibility. First, BM cells from wild type mice competitively replace that of non-irradiated *Lig4*^{Y288C} mice [12]. Thus even in the absence of stress-induced hematopoiesis, DNA damage accumulation leads to stem cell exhaustion. Second, in many cases, the phenotype of DNA repair deficient mice (*Lig4*^{Y288C}, *Csb*^{m/m}, *Xpd*^{TTD}, *Xpa*^{-/-}) is milder than that of the human diseases they model (Ligase IV syndrome, Cockayne syndrome, trichothiodystrophy, xeroderma pigmentosum complementation group A, respectively) [12,14,32,33]. For example, BM failure is a common feature in Ligase IV syndrome [34] and has been reported in xeroderma pigmentosum [35], unlike the mouse models, which do not become pancytopenic. This may indicate that mice incur less spontaneous DNA damage than humans, or that the environment is a major source of genotoxic stress [36] and the pristine conditions in which laboratory mice are kept is not a good model of humans. Cumulatively, these observations suggest that humans may be at greater risk of HSC exhaustion due to DNA damage accumulation than laboratory mice.

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Figure 1.

Schematic diagram of the linear hierarchy of hematopoietic cells. Cell surface markers used to identify each cell population are indicated with colored bars. Checked bars indicate surface markers with low expression. The blue color of the nuclei indicates the level of cell differentiation (dark blue indicating pluripotent cells; lighter blue indicating more committed cell lineages), which is correlated with proliferation rate (dark blue quiescent; lighter blue proliferating). Long term reconstituting hematopoietic stem cells (LT-HSCs) divide to produce a phenocopy of themselves (self-renewal) and a more committed multi-potent progenitor ST-HSC (short term reconstituting hematopoietic stem cell). ST-HSCs produce multi-potent

progenitor cells (MPPs), which in turn produce lineage specific common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs).

Figure 2.

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

Table I
Comparison of the hematopoietic defects in mouse models of genome instability disorders.

			Frequency of cells in the BM of mutant mice relative to wt					Competitive BM transplant					
Genotype	Age Studied	Bone marrow	L.T-HSC	ST-HSC	MPP	CLP	CMP	Donor age	Cell type [±]	STR	LTR	Reference	
G3 <i>mTR</i> ^{-/-}	13 wks and 1 yr	normocellular	nl	nl	nl	↓	↓	1 yr	HSC	↓	↓	[13]	
<i>XPD</i> ^{TD}	9 wks and 1 yr	normocellular	nl	nl	↓	↓↑*	↓	½ yr	HSC	↓	↓	[13]	
<i>Ku80</i> ^{-/-}	9 wks and ½ yr	normocellular	nl	nl	↓	↓	↓	16 wk	HSC	↓	↓	[13]	
<i>Lig4</i> ^{Δ288C}	~10 wks and ½ yr	hypocellular	nl	↓↓	↓↓			~18 wk	MNC	↓	↓	[12]	
<i>FancC</i> ^{-/-}	½ yr	normocellular	nl	nl				~7 wks or ½ yr	MNC or HSC	↓	↓	[37,38]	
<i>Brdca2</i> ^{m/m} @	adult	hypocellular	nl	nl	nl			adult	MNC	↓	↓	[24]	
<i>Msh2</i> ^{-/-}	6–8 wks	normocellular						~7 wk	MNC	nl	↓	[39]	
<i>Rad50</i> ^{ss} @	4–8 wks	BM failure						~6 wk	fetal liver	no engraftment		[22]	
<i>p53</i> ^{-/-}	16 wks	normocellular	nl						MNC	↑	↗	[25]	
<i>p53</i> ^{+/m} #	16 and 1½ yr	normocellular	↓↓					16 wk	MNC	nl	↓	[25]	
<i>Atm</i> ^{-/-}	8 wks and ½ yr	progressive BM failure		↓↓	↓↓			8 wks or ½ yr	MNC or HSC	↓↓	↓↓	[23]	

Abbreviations: G3 third generation, nl normal or no significant difference from wild type mice, HSC hematopoietic stem cells, MNC bone marrow mononuclear cells, STR short-term reconstitution of the BM; LTR long term reconstitution of the BM.

[±] the cell type isolated from DNA repair-deficient mice that was transplanted: hematopoietic stem cells (HSC) or mononuclear cells (MNC).

^{*} indicates a progressive process; the frequency of that cell population is decreased even further in old mice relative to young.

[≠] all recipients died of lymphoma within 12 wks of BM transplantation.

@ hypomorphic mutation

hyperactive mutant