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DNA Damage and Aging around the Clock

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Abstract

The hematopoietic system undergoes many changes during aging; the causes and molecular mechanisms behind these changes are not well understood. Wang *et al.* have recently implicated a circadian rhythm gene, *Per2*, as playing a role in the DNA damage response and in the expression of lymphoid genes in aged hematopoietic stem cells.

The hematopoietic system is known to undergo several changes during aging, including loss of its ability to regenerate itself, as well as diminished immunocompetence and elevated disease incidence. Many of these changes have been attributed to the altered functional potential of aged hematopoietic stem cells (HSCs); previous studies have shown that accumulation of DNA damage, transcriptional changes, epigenetic modifications and altered lineage contribution could be contributing, in part, to this change in functional potential [1] [2].

HSCs are subject to multiple sources of DNA damage, many of which are intrinsic to the cell. One contributing factor to the accumulation of DNA damage during aging is the downregulation of the DNA damage response (DDR) and repair genes associated with the quiescent nature of HSCs. Upregulation of these genes occurs when HSCs leave their quiescent state and enter the cell cycle [3]. Quiescence also helps prevent replication-associated DNA damage. Indeed, replicative stress has been implicated as a 'driver' of aging in HSCs [4]. Given the importance of maintaining genomic integrity of the stem cell population, there may be additional mechanisms in place to mitigate DNA damage accumulation in HSCs, such as expression of telomerase (for maintenance of telomere length) [5], or other novel cell type-specific molecular pathways. One such mechanism used by HSCs is the process of differentiation by which damaged stem cells are removed from the

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self-renewing population in response to DNA damage accumulation [6]. Interestingly, this response appears to be more robust in the lymphoid-biased subset of HSCs (Ly-HSCs) [6], correlating well with the skewed composition of the aged HSC compartment, which shows a significant decrease in the frequency of Ly-HSCs [7]. This, together with the overall loss of immunocompetence in aged animals, supports the hypothesis that there might be differential regulation of lineage-biased HSC subpopulations.

Wang *et al.* further explore such differential regulation of murine HSC subsets, and find that expression of lymphoid genes in Ly-HSCs is controlled – at least partially – by *Per2* (period circadian rhythm 2). *Per2* is a transcription factor that binds E-boxes and is largely studied in the mammalian brain in the context of circadian rhythms. However, E-boxes have been demonstrated to play a critical role in lymphopoiesis [8], and using an *in vivo* RNAi screen, the authors initially identified *Per2* as a gene regulating HSC potential in the context of critically short telomeres. Downregulation of *Per2* by shRNA significantly improved the functional potential of third generation (G3) telomerase-deficient *mTerc*^{-/-} mouse stem cells and progenitors (Lin⁻, Sca-1⁺, c-Kit⁺, or LSKs) which have critically short telomeres. Loss of *Per2* allowed these cells to robustly, and serially, reconstitute lethally irradiated recipients. The authors assayed the role of *Per2* in damaged HSCs (irradiated, replication stressed, or purified from physiologically aged animals) and showed that regardless of how the damage was introduced, *Per2*^{-/-} HSCs exhibited significantly improved function (including reconstitution capacity).

Given the increased functional potential of damaged *Per2*^{-/-} HSCs, the authors examined the DDR of *Per2* knockout (KO) cells. Although the number of γ H2AX and 53BP1 foci (markers of DNA damage) were not affected by *Per2* status in either young or aged HSCs, they observed a significant reduction of p-RPA (marker of replicative stress signaling) in *Per2*^{-/-} HSCs that had undergone enforced replicative stress (serial transplant or hydroxyurea treatment). Hence, these data suggest that *Per2* plays an important role in replicative stress signaling and furthermore, that the diminution of this signal may lead to the improved function of damaged HSCs. In addition to *Per2*'s role in replicative stress, the authors explored the DDR following gamma irradiation (IR). Upon IR, lineage negative (Lin⁻) *Per2*^{-/-} cells exhibited a diminished ATM kinase response that led to reduced p-CHK1, p-CHK2 and p-p53 relative to wild type cells. The loss of PER2 protein was also associated with loss of BCL2 downregulation, BAX and PUMA upregulation as well as CASP3 cleavage. Accordingly, *Per2* deletion also led to diminished survival of LSK cells whereas *Per2* overexpression resulted in increased apoptosis of Lin⁻ cells. Of note, IR induced the expression and stabilization of *Per2* mRNA and protein in Lin⁻ cells in a p53-independent manner, as Lin⁻ *p53*^{-/-} mouse cells also presented *Per2* upregulation. Consistent with these findings, while *p53*^{-/-} animals succumbed to tumors, *Per2*^{-/-} mice did not. The authors then observed that IR-induced BATF – a regulator of AP1 previously shown to regulate HSC differentiation upon DNA damage [6] – was intact in *Per2*^{-/-} HSCs, thus suggesting a potential mechanism by which *Per2*^{-/-} mice might stay tumor-free.

In addition to its role in DNA damage signaling and survival, the authors also showed that *Per2* plays a role in lineage potential. Interestingly, aged HSCs exhibited increased levels of *Per2* mRNA and protein compared to young HSCs, though aged Ly-HSCs showed a more

robust increase than My-HSCs. Moreover, deletion of *Per2* rescued the diminished lymphoid potential of aged HSCs in a cell-intrinsic manner, as demonstrated in transplantation experiments. To mechanistically explain the rescue of lymphopoietic potential in *Per2*^{-/-} HSCs, the authors analyzed the expression of lymphopoiesis-associated genes in young and aged wild type and KO Ly-HSCs. They observed increased expression of 23 of 71 lymphoid genes tested in *Per2*^{-/-} HSCs, while the age-associated decrease in the expression of many of these genes was not observed in aged *Per2*^{-/-} HSCs. To further explore the effect of *Per2* on lymphoid potential, early B lymphocytic progenitors (EPBs) from *Per2* KOs were examined. From a functional standpoint, not only did loss of *Per2* lead to increased numbers of EPBs in the aged bone marrow, aged EPBs generated more mature B-cells upon IL7 stimulation in culture. Moreover, *Per2* deletion also rescued the levels of blood IgG₁ in aged mice, concomitant with a better immune response against *Staphylococcus aureus* infection.

In summary, the study by Rudolph's group demonstrates that following DNA damage or during aging, *Per2* differentially regulates Ly-HSCs and ultimately, lymphopoiesis (Figure 1). These findings open the door to new questions, including elucidating how *Per2* modulates the DDR in the HSC compartment and how it regulates the expression of lymphoid genes. Regarding the role of *Per2* in DDR and survival, it will be interesting to see if *Per2*^{-/-} HSCs display dampened replicative stress signaling or reduced sensitivity to stress (due to reduced overall cycling) compared to *Per2*^{+/+} HSCs. The authors report a more robust IR-induced *Per2* expression upon IR or during aging in Ly-HSCs than in My-HSCs, but the analysis of downstream signaling following IR was studied only in Lin⁻ cells, due to technical limitations of obtaining sufficient cell numbers. Consequently, given the heterogeneity of the Lin⁻ population, the role of *Per2* in response to IR in primitive cells may be obscured by the response of abundant, highly replicative lineage-committed progenitor cells.

PER2's role in the regulation of lymphoid gene expression might be quite straightforward as it is a transcription factor that binds E-boxes, implicated in the modulation of genes important for lymphopoiesis [8]. Furthermore, in the brain, PER2 is associated with complexes that include the H3K9 histone methyltransferase HP1γ-Suv39h or the H3K9 histone deacetylase HDAC1 [9], chromatin marks typically associated with transcriptional repression. Given that epigenetic regulation has been previously shown to play an important role in stem cell aging [10], a putative loss of transcriptional repression stemming from PER2 deficiency might help explain, at least in part, how *Per2*^{-/-} HSCs retain the expression of genes important for the lymphoid lineage during aging. However, this mechanism has not been formally examined in HSCs.

Ultimately, this study has defined a novel role for a circadian rhythm gene, *Per2*, as a regulator of HSC survival and lineage potential, possibly providing a therapeutic target to restore lymphoid potential in an aged HSC compartment.

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References

1. Geiger H, et al. The ageing haematopoietic stem cell compartment. *Nat Rev Immunol.* 2013; 13:376–389. [PubMed: 23584423]
2. Rossi DJ, et al. Stems cells and the pathways to aging and cancer. *Cell.* 2008; 132:681–696. [PubMed: 18295583]
3. Beerman I, et al. Quiescent Hematopoietic Stem Cells Accumulate DNA Damage during Aging that Is Repaired upon Entry into Cell Cycle. *Cell Stem Cell.* 2014
4. Flach J, et al. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. *Nature.* 2014; 512:198–202. [PubMed: 25079315]
5. Morrison SJ, et al. Telomerase activity in hematopoietic cells is associated with self-renewal potential. *Immunity.* 1996; 5:207–216. [PubMed: 8808676]
6. Wang J, et al. A differentiation checkpoint limits hematopoietic stem cell self-renewal in response to DNA damage. *Cell.* 2012; 148:1001–1014. [PubMed: 22385964]
7. Beerman I, et al. Functionally distinct hematopoietic stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion. *Proc Natl Acad Sci U S A.* 2010; 107:5465–5470. [PubMed: 20304793]
8. Ephrussi A, et al. B lineage-specific interactions of an immunoglobulin enhancer with cellular factors in vivo. *Science.* 1985; 227:134–140. [PubMed: 3917574]
9. Duong HA, Weitz CJ. Temporal orchestration of repressive chromatin modifiers by circadian clock Period complexes. *Nat Struct Mol Biol.* 2014; 21:126–132. [PubMed: 24413057]
10. Beerman I, Rossi DJ. Epigenetic Control of Stem Cell Potential during Homeostasis, Aging, and Disease. *Cell Stem Cell.* 2015; 16:613–625. [PubMed: 26046761]

Figure 1. *Per2* Regulates DNA Damage Responses and Lymphoid Gene Expression during Aging or upon DNA Damage Induction

In wild type HSCs, DNA damage or aging induces the upregulation of *Per2* gene expression, and this response is more robust in lymphoid (Ly)-HSCs than in myeloid (My)-HSCs. *Per2* deficiency ($Per2^{-/-}$) in HSCs leads to a reduction both in cell survival and lymphoid gene expression, contributing to a myeloid-biased lineage output in aged animals. *Per2* deletion also results in impaired loss of HSCs upon DNA damage, contributing to the maintenance of lymphoid gene transcription in Ly-HSCs, and leading to a more balanced lineage output

during aging. E, erythrocyte; Pl, platelet; M, monocyte; G, granulocyte; T, T lymphocyte; B, B lymphocyte.p

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