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503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

Early Generation Telomerase Deficient Mice to Investigate Clonal Hematopoiesis in Aging

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Clonal hematopoiesis is an age-associated condition characterized by the expansion of hematopoietic stem cell (HSC) pools with somatic mutations, observed in 10-30% of individuals older than 70 years. This phenomenon is primarily driven by mutations in transcriptional regulators such as DNMT3A, TET2, and ASXL1. While the clonal expansion of mutated HSCs is ubiquitous with age, it appears largely benign in the absence of strong selective pressures. These benign clones might compensate for the loss of regenerative HSC capacity during aging, and skew differentiation towards the myeloid lineage. Telomere erosion plays a significant role in this process. Telomeres normally prevent the activation of the DNA damage response (DDR) machinery. However, with each cell division, telomeres shorten until they reach a critical length, eventually leading to apoptosis or senescence. Previous studies have suggested a potential involvement of telomere attrition in the development of clonal hematopoiesis (Hinds et al. Blood 2016; Zink et al. Blood 2017). Late generation telomerase deficient (Terc-/- G5) mice develop premature bone marrow failure and mortality, however earlier generation Terc-/- G3 (3 homozygous crosses) have shortened telomeres but appear relatively normal. Importantly, inhibitors of telomerase have recently entered clinical trials for haematological malignancies and therefore understanding the long-term effects of telomerase inhibition is important. By studying the interplay between telomere attrition, HSC aging, and the development of CHIP, we aim to gain insights into the mechanisms underlying age-associated hematopoietic changes and their potential clinical implications.

We examined young (age 8-10 week) and aged (10-12 month) Terc-/- G3 mice. Telomere lengths were significantly reduced in bone marrow cells from young Terc G3 (15 kb) when compared to wild-type mice (30 kb) with mild reduction in BM cellularity. Absolute long-term HSC numbers were reduced, as were megakaryocyte erythroid progenitors, but there was preservation of granulocyte macrophage progenitors and common myeloid progenitors. Spleen progenitor numbers were not affected. In comparison, aged (10-12 months old) Terc G3 mice showed enhanced differentiation skewing in the peripheral blood with significantly increased myeloid ($n = 8$ wt; $n = 12$ Terc ko; $p = 0.0004$) and concomitantly reduced B cell ($p = 0.0002$) and T cell ($p = 0.0320$) populations. There was marked mobilization of bone marrow progenitors to the spleen, most notably GMP ($p = 0.0004$) but also MEP and CMP in aged Terc G3. Furthermore, aged Terc G3 ko now exhibited marked reduction in bone marrow CMP ($p < 0.0001$) and MEP ($p < 0.0001$), but skewing towards GMP ($p<0.001$) resulting in normal total GMP numbers, but markedly reduced CMP and MEP numbers ($p < 0.0001$). Compared to young Terc G3 mice, the aged Terc G3 mice had relative expansion of the BM LT-HSC population, and therefore normal numbers of BM LT-HSC compared to aged matched controls. Aged Terc G3 BM LTHSC have been isolated for gene expression and clonality analysis. Together, these findings are consistent with accelerated aging in G3 Terc-/- mice, providing a model for examining clonal hematopoiesis.

In order to assess the cell intrinsic function of Terc G3 LTHSC, we next performed non-competitive bone marrow transplantation experiments from Terc ko or wild-type donors into lethally irradiated young wild-type recipients ($n = 9$ per group), and analysed these at 4 months post-transplant. There was reduced, but stable reconstitution by Terc G3 cells, consistent with previous competitive bone marrow transplants in Terc G3 (Bruedigam et al. Cell Stem Cell 2014). We observed a similar reduction in bone marrow cellularity in Terc ko transplanted recipients ($p = 0.003$). Lineage skewing was less pronounced but CMP percentages were significantly reduced in Terc ko compared to wild-type conditions ($p = 0.03$). In contrast to primary mice, mobilization of progenitors to the spleen was not observed, suggesting that this finding was ameliorated by normal bone marrow and splenic supporting cells.

The aged G3 Terc ko model faithfully recapitulates the haematopoietic phenotype of aging in humans and we propose to utilise this model to further our understanding of the mechanisms and physiological consequences of clonal hematopoiesis, to eventually help identify strategies to mitigate its clinical consequences.

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