DO HEMATOPOIETIC STEM CELLS GET OLD?

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Published in Leukemia 31, 529-531 (2017)
In many countries of the world the proportion of elderly people will rise very substantially in the upcoming decades. As a result, the number of patients that present with age-related diseases will also increase. This relates to neurodegenerative conditions such as Alzheimer’s disease that many people will instantly link to an aging society, but it also includes multiple hematological syndromes that display clear increases in incidence with advanced age (Figure 1). Whereas in the United States for a long time the leading cause of death has been heart disease, this was recently replaced by cancer (Heron M, 2016). More old people will result in more patients with leukemia and in increasing health care costs for their treatment (Figure 2).

![Figure 1: Observed incidence of several hematological diseases by age in the Netherlands in 2015. Source: Netherlands Cancer Registry, managed by IKNL © June 2016.](image)

In addition to these clear-cut hematological diseases, there are multiple other (pre)-clinical manifestations that may be affected by malfunctioning of the hematopoietic system. These include for example an increased susceptibility to infections (due to reduced numbers and functioning of lymphocytes) (Frasca et al., 2008), reduced vaccination efficiency (Goodwin et al., 2006) and an increased risk of arteriosclerosis (due to altered macrophage activity), anemia (Tettamanti et al., 2010), and maybe even some neurological conditions (as a result of loss of microglia functioning) (Mosher and Wyss-Coray, 2014).

To explore why many hematological diseases occur much more frequently in older people, we first need to assess what changes with age in blood (precursor) cells. Functionally, hematopoietic stem cells produce
It appears very likely writers and erasers have been shown to play important roles in not properly copied to the daughter cells, which would result in an age-dependent detrimental stem cell phenotypes have been reported. Most notably, the levels of engraftment upon transplantation of a single, or a low number, of purified hematopoietic stem cells are much lower when the donor cells originate from an old mouse, compared to young cells (Dykstra et al., 2011). These data strongly suggest that the number of mature cells produced per stem cell declines with age. However, it is not only the absolute number of mature produced cells that is declining, aging is also associated with lineage-skewing, which refers to the observation that the relative proportion myeloid and lymphoid cells changes in favor of myeloid cell production (Beerman et al., 2010).

It has not been very well studied to what extent the functional activity of mature, fully differentiated, blood cells such as erythrocytes, platelets, granulocytes and macrophages is reduced upon normal aging.

Most of the above observations have been made in mouse models, and although there is little reason to believe that human hematopoietic stem cells age differently compared to those in mice, it is important to note fewer progeny as they age. This has been best studied in mice. While it is clear that old mice do not run out of stem cells, and indeed classical serial transplantation studies have documented that hematopoietic stem cells can outlive their original donor mouse (Harrison, 1979), many age-dependent detrimental stem cell phenotypes have been reported. It is interesting to note that the clonal hematopoiesis that accumulated with each of these cell divisions, it seems very well considered preleukemic, either due to the fact that the mutation increases its proliferative activity and thus the odds that this preleukemic cell is hit by a second oncogenic event, or alternatively, the pathological consequences compared with if it had occurred in an epigenetically unperturbed cell.

If reprogramming indeed is able to reverse hematopoietic cell transcriptome must be carefully controlled by the collective or DNA demethylating agents, that inhibit specific epigenetic pathways by exposing random genetic mutations (which obviously would not be corrected during reprogramming).

Figure 2:
Cost of leukemia care by phase of care in male and female patients older than 65, in 2010 US dollars.
that we have not fully assessed to what extent aging of hematopoietic stem cells is evolutionary conserved in these two species.

What is also largely unknown is the extent to which aging of stem cells is conserved across multiple regenerating tissues. Although intuitively one would expect that mechanisms that contribute to stem cell aging may be operating in all adult stem cell populations, it is also possible that major differences exist. For example, where it is generally believed that hematopoietic stem cells are normally largely quiescent, and in fact stem cell activation is believed to be detrimental (Walter et al., 2015), in the intestinal system this appears to be quite the opposite. Intestinal stem cells have been reported to cycle very actively, yet these cells seem to be exempt from the aging process (Clevers, 2013). In muscle stem cells, in contrast, several aging characteristics that are observed in the hematopoietic system appear to be also present (Brack et al., 2007). If the mechanisms that contribute to aging are conserved in multiple tissues, it is conceivable that interventions to prevent stem cell aging in one tissue may in fact also affect those in others.

We hypothesize that the age-dependent loss of hematopoietic homeostasis finds its origin in detrimental molecular events that first occur in primitive hematopoietic stem cells. The impaired ability of aged hematopoietic stem cells to properly balance the choice between self-renewal and differentiation may predispose to hematological and -possibly- other disorders. Therefore, efforts to prevent such age-dependent hematopoietic stem cell deterioration are expected to be beneficial at multiple levels.

Our understanding of the molecular causes that underly stem cell aging is still limited. A great unknown is whether impaired functioning of hematopoietic stem cells results from cell-intrinsic or rather cell-extrinsic causes. This is not only of academic interest but is also highly relevant if approaches are developed to delay, prevent, or indeed reverse, stem cell aging. What would be the cell type to target, the stem cell itself, or the microenvironmental niche cell next to which it lives? Experimental transplantation studies have shown that transplanting old stem cells in a young recipient does not erase functional decline, strongly suggesting that at least a major component that contributes to stem cell aging must be a cell-intrinsic feature (Rossi et al., 2005). However, it is also very clear that the constitution of aged bone marrow is very different compared to young. In aged human bone marrow adipogenesis is much more prevalent than in young, and bones become very brittle upon aging (Rozman
et al., 1989). The molecular and cellular composition of the bone marrow microenvironment, which contains the elusive hematopoietic stem cell niche, has only recently been studied in significant detail (Birbrair and Frenette, 2016) and at current it is far from clear how this microenvironment changes during aging, and how this might contribute to decreased stem cell functioning.

An interesting observation in elderly humans is that the hematopoietic system appears to become more clonal, i.e. leukocytes in the peripheral blood are derived from fewer and fewer stem cells. Initial observations on increased clonal hematopoiesis were based on skewed X-inactivation patterns in elderly females (Busque et al., 1996), but more recently the same phenomenon has been observed using whole genome sequencing approaches (Genovese et al., 2014; Xie et al., 2014). At current it remains unclear whether oligoclonal hematopoiesis is of any clinical relevance. Several reports document very significant clonal dominance in normal elderly people, without any signs of hematological disease (Busque et al., 2012; Jaiswal et al., 2014; van den Akker et al., 2016). In experimental settings it has never been documented that mice that were transplanted with a single, or very few stem cells were more prone to develop hematological disorders.

It is of great interest to assess to what extent stem cell-intrinsic aging parameters may be reversible. An interesting experimental approach showed that hematopoietic stem cells derived from iPS cells generated from aged HSCs, were functionally equivalent to cells derived from iPS cells generated from young HSCs (Wahlestedt et al., 2013). This strongly suggests that at least a major part of the stem cell intrinsic age-dependent decline can be reversed. This also suggest that although aged HSCs appear to display increased levels of DNA damage (Beerman et al., 2014), and indeed DNA repair deficient mice and human show bone marrow pathology (Salob et al., 1992; Zhang et al., 2011), dysfunctioning of normal stem cells during aging is unlikely to result from an accumulation of random genetic mutations (which obviously would not be corrected during reprogramming).

If reprogramming indeed is able to reverse hematopoietic stem cell aging, it seems plausible that epigenetic mechanisms contribute to stem cell aging. Many studies in the last decade have demonstrated that, like any adult cell type, hematopoietic stem cells show quite a distinct gene expression profile. This stem cell transcriptome must be carefully controlled by the collective consequences of a multitude of epigenetic
modifications that compact or relax locally the stem cell genome. Many epigenetic writers and erasers have been shown to play important roles in hematopoietic stem cell activity. This includes for example Ezh2 (Kamminga et al., 2006), Bmi1 (Rizo et al., 2008; Rizo et al., 2009), Cbx7 (Klauke et al., 2013) and Dnmt3a (Challen et al., 2012). It appears very likely that upon a single stem cell division, some of the activating or repressing marks that are deposited or read by these proteins are not properly copied to the daughter cells, which would result in an aberrant i.e., stem cell incompatible, gene expression pattern, and thus to loss of stem cell functionality. We envision that such erosion from a stem cell gene expression profile is not a digital -all or nothing- event, but rather occurs very gradually every time a cell divides.

Due to the unavoidable epigenetic differences that accumulate with each of these cell divisions, it seems very well possible that old hematopoietic stem cells require quantitatively or qualitatively different mitogenic signals from their environment compared to young stem cells. It has been well documented in mice and man that hematopoietic stem cells from fetal liver, cord blood, or newly borns are fundamentally different from adult stem cells (Bowie et al., 2007; Rebel et al., 1996). Some of the molecular circuitry that is associated with these stage/age specific signals has been elucidated (Copley et al., 2013; Kim et al., 2007; Rossi et al., 2005), but it is likely that much remains to be explored. Clinically, this may be of interest when cord blood-derived stem cells in the future will be used to transplant aged recipients. Will these heterochronic transplants cause problems?

In contrast to genetic lesions, aberrant epigenetic modifications can in principle be erased and corrected. This would offer ways to reverse aspects of the aging process. The most profound example of such epigenetic resetting is obviously exemplified by the process of reprogramming adult cells to pluripotency by overexpression of several transcription factors. However, it is also possible to interfere in specific epigenetic pathways by exposing cells to (small) molecules such as histone deacetylase inhibitors or DNA demethylating agents that inhibit specific epigenetic enzymes.

It is interesting to note that the clonal hematopoiesis that is observed in a sizeable fraction of elderly people often is associated with mutations in DNMT3A (Xie et al., 2014). Also, mutations in several other epigenetic genes, including EZH2 (Morin et al., 2010) and TET2 (Kandoth et al., 2013; Langemeijer et al., 2011), are frequently found in hematological...
disorders. This suggests that mutations in genes coding for epigenetic enzymes may confer a proliferative advantage to a cell (Jung et al., 2016). Such a mutant cell may be considered preleukemic, either due to the fact that the first mutation increases its proliferative activity and thus the odds that this preleukemic cell is hit by a second oncogenic event, or alternatively, the first epigenetic mutation may change the epigenetic landscape of a preleukemic cell in such a way that a second mutation is more oncogenic compared to if it had first occurred in an epigenetically unperturbed cell.

This latter scenario should be testable and would predict that a similar oncogenic insult in an old stem cell would have different pathological consequences compared to if it had first occurred in a young cell. There is actually evidence for this; enforced overexpression of Bcr-abl in old stem cells causes different disease kinetics compared to overexpression in young stem cells (Signer et al., 2007). Thus, it is conceivable that leukemia that originates in the elderly is molecularly and functionally distinct from leukemia in young adults.

We expect that many of the questions raised above will be answered in the not too distant future, as more and more laboratories have become interested in the fundamental question as to how a self-renewing stem cell ages.

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