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Previews

Noncanonical Wnt Comes of Age in Hematopoietic Stem Cells

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Understanding molecular mechanisms of aging is crucial in efforts to reverse it. In a recent issue of *Nature*, Florian et al. (2013) report that increasing levels of noncanonical Wnt signaling accompany hematopoietic stem cell (HSC) aging, which can be modulated to functionally rejuvenate HSCs.

In many regions of our planet, the human population is aging, providing an important societal incentive for biomedical researchers to identify the molecular mechanisms that contribute to the aging process and to explore ways to halt or reverse it. Organismal aging is associated with decreased organ function and regeneration, resulting in increased morbidity and mortality. In the hematopoietic system, aging is associated with the development of immunodeficiencies, anemias, and an elevated risk of malignancies. A growing body of data suggests that these phenotypes result from changes in the pool of HSCs that are responsible for lifelong production of all blood and immune cells. The possibility to rejuvenate stem cells holds promise to reset the aging clock. Recent studies in heart, muscle, skin, and blood show that changes in signaling pathways (Wnt, Notch, TGF-β, NFκB, and mTOR) may underlie (stem) cell aging, and, excitingly, modulation of these pathways may restore tissues to a more youthful state (Loffredo et al., 2013; Rando and Chang, 2012; Signer and Morrison, 2013). Now, a report of Florian et al. suggests that activation of noncanonical Wnt signaling in HSCs is responsible for reversible age-related deterioration in the hematopoietic system (Florian et al., 2013).

The hallmarks of aging of HSCs include: (1) increased HSC pool size; (2) skewing of hematopoiesis toward the myeloid lineage and an increase of myeloid-dominant HSCs in the HSC pool; and (3) decreased repopulation potential per HSC (Figure 1) (Dykstra et al., 2011; Geiger et al., 2013). Moreover, HSCs from old mice demonstrate a reduced ability to home and engraft bone marrow niches, and they show increased mobilization in response to cytokines (Dykstra et al., 2011; Geiger et al., 2013). Although the phenotype of aged HSCs has now been well described, the molecular mechanisms that contribute to stem cell aging are not well understood. HSC aging has been associated with an upregulation of myeloid and a concomitant downregulation of lymphoid genes, increased production of reactive oxygen species, elevated levels of DNA damage, and an activation of proinflammatory molecules (Geiger et al., 2013). Recently, loss of cell polarity, modulated by the small Rho GTPase Cdc42, in old HSCs has been described as a novel aging mechanism (Florian et al., 2012). In a recent issue of *Nature*, the same group now proposes that the underlying cause of HSC aging is a shift from canonical to noncanonical Wnt signaling (Florian et al., 2013).

The Wnt pathway is an evolutionarily conserved signaling cascade that regulates a wide variety of processes, including embryonic organogenesis and stem cell proliferation in adults. The Wnt signaling network is highly complex: 19 secreted Wnt proteins have been described in mammals, all of which together can bind to more than 15 receptors and coreceptors (Niehrs, 2012). Wnt can act in both an autocrine and paracrine fashion. The best-studied (canonical) Wnt signaling pathway is β-catenin dependent and results in activation of target genes of the transcription factor family Tcf/Lef. Wnt pathways that do not rely on β-catenin are cumulatively referred to as noncanonical, and they have been shown to be important for establishment of cell polarity and cell migration. While binding of Wnt protein to its receptor and coreceptor determines which downstream pathway is induced, some Wnt ligands (Wnt1, Wnt3A, and Wnt8) have been associated with predominantly canonical signaling while others (Wnt5A and Wnt11) appear involved in noncanonical signaling (Niehrs, 2012). Noncanonical Wnt activation inhibits the canonical pathway, at least partially as a result of competition for receptor binding. In the hematopoietic system, the role of Wnt signaling has thus far been controversial (Luis et al., 2012). For instance, studies modulating canonical Wnt activity report pronounced augmentation but also total exhaustion of HSC function in response to β-catenin overexpression (Luis et al., 2012). It has been proposed that levels of Wnt signaling are tightly regulated and dose-dependently affect HSCs (Luis et al., 2012).

The current study of Florian et al. describes a new role for the Wnt cascade in HSCs and suggests that the balance between canonical and noncanonical Wnt ligand expression, and therefore Wnt signaling, underlies HSC aging (Florian et al., 2013). The authors show that the levels of Wnt5a are significantly elevated in aged long-term HSCs (LT-HSCs), while expression of canonical Wnt ligands remains unaltered during aging (Figure 1B).

Previously, Cdc42, a downstream effector of noncanonical Wnt signaling, had been associated with HSC aging by this same group (Florian et al., 2012). In aged HSCs, elevated levels of Cdc42 are associated with cell depolarization, in contrast to predominantly polarized distribution of tubulin and Cdc42 in young HSCs (Florian et al., 2012). Now, the authors report that the number of

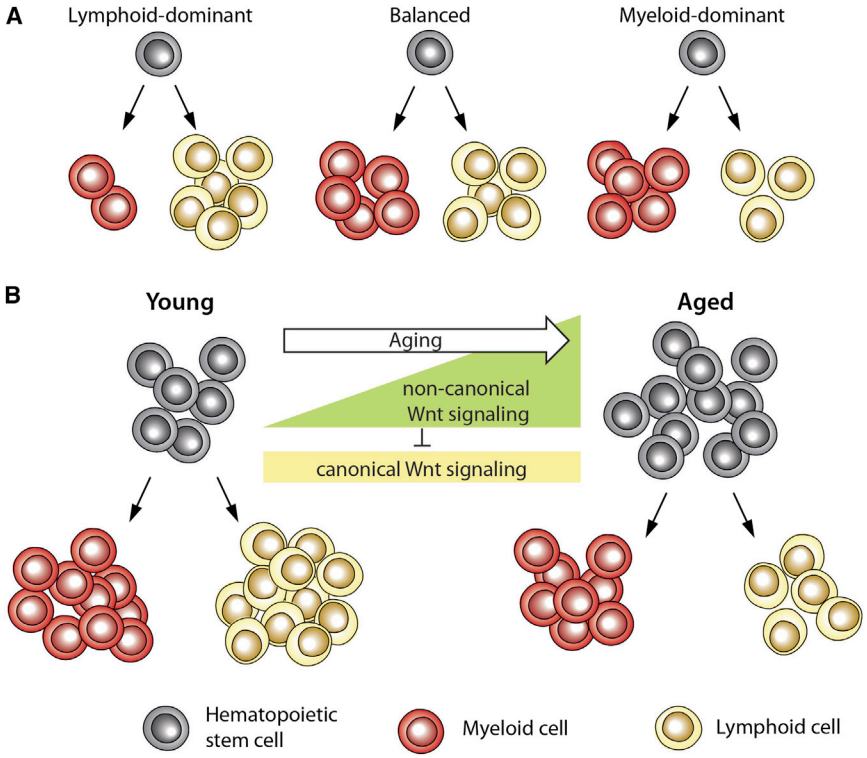

Figure 1. Changes in Wnt Signaling Accompany Aging in the HSC Pool

(A) Heterogeneity of the developmental potential of HSCs. Based on their ability to produce differentiated output, HSCs at the single-cell level can be classified as balanced, lymphoid-dominant, or myeloid-dominant.

(B) Changes in the HSC pool upon aging. During aging, the number of phenotypically defined HSCs increases dramatically, whereas both the frequency of functional HSCs within the putative pool and the repopulation capacity per HSC decreases. Production of mature cells becomes skewed toward the myeloid lineage. The levels of canonical Wnt proteins remain constant over the life span, while the levels of noncanonical Wnt proteins increase, leading to inhibition of canonical signaling.

polarized HSCs is negatively correlated with Wnt5a levels (Florian et al., 2013). Increasing or decreasing Wnt5a levels leads to induction or reversal of age-related HSC phenotypes, respectively. Exposure of HSCs to Wnt5a induced lymphoid to myeloid skewing of donor cells in blood after transplantation, while conversely, Wnt5a downregulation increased lymphoid chimerism from aged HSCs. Moreover, the age-related increase of the fraction of phenotypically defined LT-HSCs was attenuated in aged $Wnt5a^{+/-}$ mice, and a similar effect was observed in recipients of aged Wnt5a shRNA-transduced bone marrow cells.

While these data provide exciting support for the notion that age-related HSC changes can be attenuated by alteration of signaling, several important questions remain. It has now become evident that individual HSCs are highly heterogeneous in their turnover rate and their developmental and repopulating potential (Figure 1). The ratio between these distinct types of HSCs shifts with age and is reflected in changes of cellular constituents in blood. The experiments by Florian et al. were performed using populations of cells, and the effect of Wnt5a on different individual HSCs cannot be distinguished. Therefore, an alternative explanation of the observed reversal of myeloid/lymphoid skewing upon Wnt5a downregulation is preferential survival of balanced/lymphoid-dominant cells upon treatment. Moreover, one of the key features of HSC aging is a strongly decreased self-renewal and repopulation capacity in phenotypically defined individual HSCs. It remains unclear whether inhibition of Wnt5a in aged HSCs results in improved reconstitution ability in primary and secondary recipients, and whether the frequency of functional HSCs is increased. It is striking that the aging HSC phenotype could be induced by only a 16 hr incubation period with Wnt5a ligand. As biological aging is proposed to be at least partly mediated by changes in the epigenome (Rando and Chang, 2012), it is remarkable that an exposure period shorter than one cell division can have such a profound effect.

The experiments by Florian et al. (2013) provide a novel perspective on hematopoietic stem cell aging. The studies suggest that potentially reversible cell-intrinsic mechanisms are involved in this process. For a clear understanding of such rejuvenation process, it will be essential to uncover additional mechanisms and the kinetics of changes of the epigenetic landscape that coincide with aging.

REFERENCES

Dykstra, B., Olthof, S., Schreuder, J., Ritsema, M., and de Haan, G. (2011). J. Exp. Med. *208*, 2691–2703.

Florian, M.C., Dörr, K., Niebel, A., Daria, D., Schrezenmeier, H., Rojewski, M., Filippi, M.D., Hasenberg, A., Gunzer, M., Scharffetter-Kochanek, K., et al. (2012). Cell Stem Cell *10*, 520–530.

Florian, M.C., Nattamai, K.J., Dörr, K., Marka, G., Uberle, B., Vas, V., Eckl, C., Andrä, I., Schiemann, M., Oostendorp, R.A., et al. (2013). Nature, in press. Published online October 20, 2013. http://dx.doi.org/10.1038/nature12631.

Geiger, H., de Haan, G., and Florian, M.C. (2013). Nat. Rev. Immunol. *13*, 376–389.

Loffredo, F.S., Steinhauser, M.L., Jay, S.M., Gannon, J., Pancoast, J.R., Yalamanchi, P., Sinha, M., Dall'Osso, C., Khong, D., Shadrach, J.L., et al. (2013). Cell *153*, 828–839.

Luis, T.C., Killmann, N.M., and Staal, F.J. (2012). Leukemia *26*, 86–90.

Niehrs, C. (2012). Nat. Rev. Mol. Cell Biol. *13*, 767–779.

Rando, T.A., and Chang, H.Y. (2012). Cell *148*, 46–57.

Signer, R.A., and Morrison, S.J. (2013). Cell Stem Cell *12*, 152–165.