Commentary

Stem Cell INVESTIGATION

Maintenance of hematopoietic stem cell dormancy: yet another role for the macrophage

Karin Gustafsson[1,2], Michael Welsh[3]

[1]Department of Stem and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA; [2]Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA 02114, USA; [3]Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

Correspondence to: Michael Welsh. Department of Medical Cell Biology, Uppsala University, Husargatan 3, Box 571, 75123, Uppsala, Sweden. Email: Michael.welsh@mcb.uu.se; Karin Gustafsson. MGH Center for Regenerative Medicine, 185 Cambridge Street, CPZN 4400, Boston, MA 02114, USA. Email: UGUSTAFSSON@mgh.harvard.edu.

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Hematopoietic stem cells (HSC) possess several unique features that ensure a life-long supply of all bone marrow derived blood cell lineages (1). In adulthood, they are mainly localized to the bone marrow and depend on a supportive niche for maintaining their long-term (LT) repopulating ability while simultaneously having a capacity for multi-potent differentiation. The HSC niche has been suggested to be composed of vascular endothelial cells, perivascular mesenchymal stromal cells (2-4), osteoblasts (5) as well as mature hematopoietic cells like megakaryocytes and macrophages (6-9). Furthermore, HSC maintain a low cell cycle activity to prevent exhaustion of their replicative capacity (10). In addition, recent findings suggest that HSC can be subdivided into LT or short-term (ST) HSC of which the latter have a greater propensity for lineage-specific differentiation (11). Mouse LT-HSCs are enriched in a cell population immunophenotypically characterized as Lin^- (lineage negative), c-Kit^+, $Sca1^+$, $CD150^+$, $CD34^-$, Flk2/$Flt3^-$ and $CD48^-$ cells. A recent study adds expression of Hoxb5 to these markers for providing additional definition of the LT-HSC population (12).

The study by Hur *et al.* (13) identifies yet another marker for LT-HSC and furthermore ascribes a mechanistic role to this marker. Cd82/Kai1 codes for a membrane receptor (denoted CD82 onwards) belonging to the tetraspanin family of receptors, which interacts with the Darc/Cd234 gene product (denoted Darc onwards) (14). Cd82 is ubiquitously expressed but in the bone marrow, high Cd82 expression is mainly confined to LT-HSC located in endosteal and/or periarteriolar compartments. During hematopoietic differentiation, Cd82/CD82 expression decreases being significantly lower in ST-HSC and multi-potent progenitors. Knockout of Cd82 diminished the population of LT-HSC while simultaneously increasing their proliferation via cell cycle entry. This resulted in a decreased repopulating capacity when competitively transplanted to bone marrow ablated donors, an effect that became increasingly apparent upon performing serial bone marrow transplantations. Surprisingly, this effect was less pronounced when assessing the reconstitution of the myeloid lineage only. Mechanistically, based on *in vitro* knockdown experiments, CD82 via protein kinase C alpha (PKCa) increases the expression of Tgfb1 and Tgfbr1 that via Smad signaling elevate the expression of cyclin dependent kinase inhibitors (CKI), thus maintaining LT-HSC in a quiescent state.

The cellular source of the CD82 binding partner Darc was next established. Highest bone marrow Darc expression was localized to macrophages that were in direct contact with quiescent LT-HSC. In LT-HSC and $Darc^+$ macrophage co-culture experiments, $Darc^+$ macrophages increased LT-HSC quiescence in a Tgfb1 and Smad3-dependent manner. Additional mechanistic twists delineated in the study were that Darc was required for LT-HSC CD82 expression by prevention of endocytic degradation of CD82 and that Darc-CD82 associations also are operating in maintaining human LT-HSC quiescence.

In summary, a model is proposed suggesting that macrophages via Darc expression increase/retain LT-HSC expression of CD82 and that CD82-signaling via

PKCa/Tgfb1/Tgfbr1/Smad3/CKI results in LT-HSC quiescence. Absence of this pathway will eventually deplete the bone marrow of LT-HSC. This pathway may also assist in maintaining physiological hematopoiesis since under physiological steady state conditions, Darc-activated CD82 suppresses LT-HSC proliferation, whereas when the bone marrow is challenged by genotoxic stress, the suppressive effect of CD82 is lost due to the simultaneous disappearance of Darc-expressing macrophages.

The study reveals certain consequences of perturbed bone marrow niche/LT-HSC interactions as a result of CD82 ablation that deserve mentioning. One is that despite increased proliferation, LT-HSC numbers are reduced without a concomitant increase of progenitor populations. Secondly, the loss of lineage repopulation was unequal with a more severe reduction of T and B cell reconstitution than that of myeloid cells. The intricacy in the behavior of LT-HSC in response to aberrant cell cycle regulation can be illustrated by the fact that in two different situations, one due to absence of CD82 (13) and the other due to absence of Shb (15), both revealed reduced numbers of bone marrow LT-HSC and decreased repopulation upon bone marrow reconstitution after transplantation, despite one being due to excessive proliferation and exhaustion of the LT-HSC pool whereas the other was due to increased quiescence caused by cell cycle inhibition. The mechanism behind the reduced proliferation in the absence of Shb is not known but may involve increased expression of the CKI $p27^{Kip1}$ (Gustafsson and Welsh, unpublished observations), which functionally relates that effect to TGF-beta signaling in LT-HSC. Several recent publications have made a strong case for TGF-beta supporting LT-HSC dormancy and most focus has been on increased expression of the CKIs $p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$ via SMAD2/3 activation (16-21). Also of possible relevance is non-canonical signaling inhibiting the PI3-kinase/Akt/FoxO pathway that could exert an effect in this context as well. Taken together, a common denominator in most situations maintaining LT-HSC quiescence could be effects relating to some step in the TGF-beta pathway conferring cell cycle inhibition.

This work also highlights the importance of the bone marrow microenvironment as a protector of LT-HSC integrity. This may, in turn, be one of the main physiological functions of the hematopoietic niche. A number of recent studies suggest that LT-HSC are not the main providers of hematopoiesis under homeostatic conditions in adulthood. Instead it appears as though ST-HSC as well as more differentiated progenitors with self-renewing abilities are the predominant suppliers of new blood cells (11,22), implying that LT-HSC primarily serve as a reservoir in the adult hematopoietic system. In addition, current data suggest that commitment to a specific lineage during the hematopoietic differentiation process, starting with ST-HSC, primarily reflects cell-autonomous features of the relevant cell type (23-26). The primary role of the LT-HSC niche may thus be to enforce quiescence in order to protect the stem cell pool from proliferative exhaustion and accumulation of DNA damage.

Macrophages have already been implicated in LT-HSC regulation through control of mobilization (6,8). We can now add another mechanism, i.e., macrophages and Darc/CD82 signaling, that participates in niche-dependent maintenance of LT-HSC self-renewal. The figure illustrates demonstrated bone marrow niche/LT-HSC interactions that play a role in LT-HSC maintenance (*Figure 1*).

The role of CD82 in acute myeloid leukemia (AML) has been investigated in a number of studies (29-33). However, none of these relates to high Cd82 expression imposing dormancy on the leukemic cells but rather suggest that CD82 promotes leukemic cell survival and bone marrow homing. This is in contrast to the situation in solid tumors in which CD82 inhibits tumor invasiveness and metastasis, which are effects more in line with the effect on dormancy of LT-HSC (16). The present study stimulates speculation on an additional involvement of CD82 in AML, proposing that leukemic stem cells with high CD82 are more quiescent and thus more resistant to chemotherapy. Future studies will address this important topic.

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Footnote

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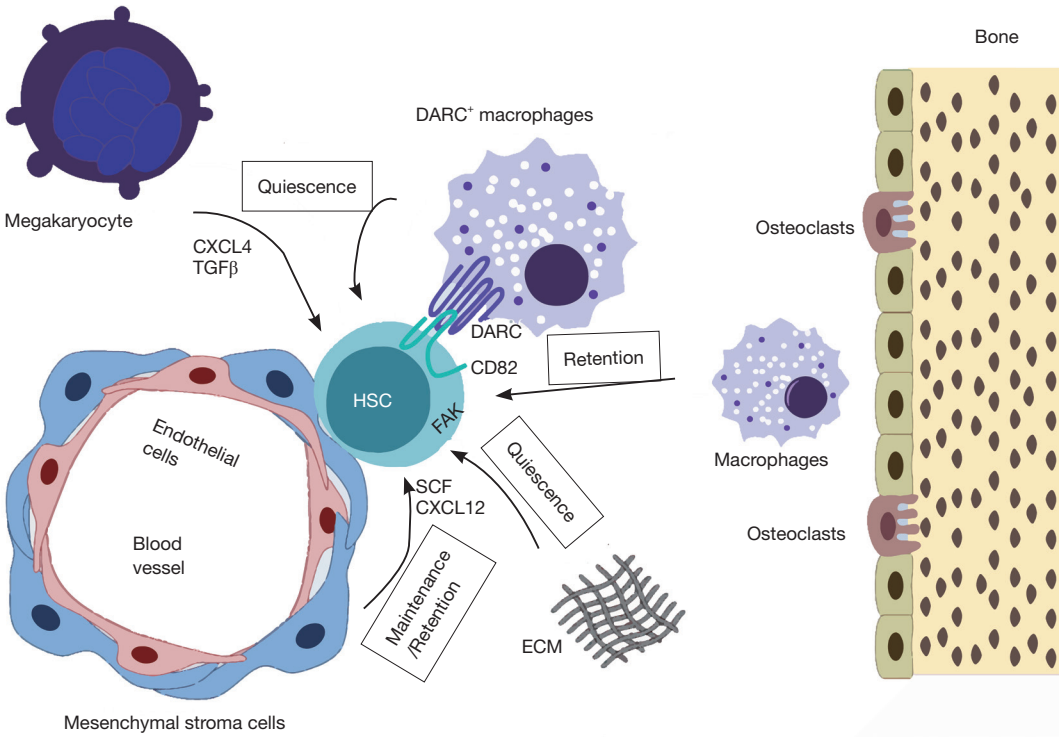

Figure 1 Schematic illustration of niche signals maintaining LT-HSC quiescence and differentiation. Signals derived from various cell types such as macrophages, endothelial cells, mesenchymal stromal cells, megakaryocytes and osteoblasts. These have all been suggested to participate in the regulation of LT-HSC dormancy and retention. The cues modulating these processes can be exerted by cell-cell contacts (as is the case in the present study on Darc/CD82), through secretion of cytokines/chemokines (2,4,7,9) and extracellular matrix (ECM) depositions. ECM will regulate focal adhesion kinase (FAK), which suppresses LT-HSC proliferation (15,27). On the other hand, in BCR-ABL-induced myeloid leukemia, increased FAK activity promotes leukemic cell proliferation (28).

stem cells through interaction with DARC-expressing macrophages. Cell Stem Cell 2016;18:508-21.

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