Commentary

Stem Cell INVESTIGATION

Shedding light on HSC dormancy—a role for the DARC

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Hematopoietic stem cell (HSC) quiescence is vital for lifelong hematopoiesis as it maintains long-term potential and self-renewal capacity of HSCs. Hypoxia is one feature of the bone marrow (BM) microenvironment thought to contribute to quiescence by limiting oxidative stress and initiating a distinct metabolic program (1). Based on their prior identification of a hypoxia response element in the promoter region of the tetraspanin CD82 gene (2), Hur and colleagues sought to address the role of this molecule in HSC function (3). Consistent with hypoxia inducible factor 1 (HIF-1α) expression (4), long term-HSCs (LT-HSCs) were found to be the exclusive primitive hematopoietic cell type expressing CD82. Expression of CD82 initiated a dormancy-enforcing program dependent upon TGFβ signaling. Furthermore, CD82 expression on HSCs was maintained by a subset of macrophages (MΦs), an emerging niche cell for HSCs. Hematopoietic demands that occur as a result of injury or infection must interrupt dormancy-enforcing programs. As MΦs are important immune sentinels, the study suggests MΦs may directly control HSC function in demand-adapted hematopoiesis and in conditions of acute or chronic inflammation (5). Thus, the insights provided by Hur *et al*. have broad implications for understanding HSC function in demand-adapted hematopoiesis, as well as aging and cancer.

Novel marker of HSC quiescence

Though numerous phenotypic markers for HCSs exist, few are linked directly to a functional attribute, such as dormancy. The observation that CD82 expression was exclusive to the pool of LT-HSCs in both mice and humans, and was linked functionally to reduced HSC cycling, indicates its use for identifying quiescent HSCs. CD82 (also referred to as KAI1) is a member of the family of tetraspanins, a highly conserved family of integral membrane proteins involved in cell adhesion, membrane trafficking, and signaling [reviewed in (6)]. Tetraspanins can both promote and suppress cancer metastases owing to their ability to modulate proliferation, migration, and senescence. CD82 was found to suppress metastases via its interaction with Duffy antigen receptor for chemokines (DARC), present on vascular endothelium (7). Indeed, solid tumors that lack CD82 expression have been shown to be more metastatic and proliferative (8,9). HSC quiescence does not absolutely require CD82, however, as a portion of HSCs remains in G_0 in the absence of CD82, and mice lacking CD82 do not exhibit signs of hematopoietic dysfunction even at 18 months (10). In fact, only about one-third of the pool of phenotypic LT-HSCs (or SLAM-HSCs) expresses CD82. It will be interesting to compare $CD82^+$ and $CD82^-$ HSCs from wild-type mice to determine whether a functional gradient exists among the HSC pool potentially regulated by CD82 expression. Nonetheless, in the absence of CD82, there was a specific reduction in LT-HSCs and an increased number of proliferating HSCs, while more differentiated progenitors were spared and comparable to wild type counterparts. So, how does CD82 enforce dormancy? To address this question a series of *in vitro* experiments was performed utilizing a progenitor cell line wherein CD82 was either overexpressed or knocked down. Cell cycle dependent kinase inhibitors were reduced upon CD82 knockdown, and expression of TGFβ signaling components, TGFβ1 and TGFβR2, were diminished by both gene expression and protein. CD82-mediated TGFβ1 expression, and maintenance of G_0, was inhibited by blocking PKCα, a molecule previously shown to associate with CD82 (11). In line with the function of CD82 in promoting LT-HSC dormancy, the tetraspanin CD81 is necessary for

preserving HSCs under myeloablative stress by promoting reentry into dormancy upon proliferation (12).

CD82-mediated maintenance of dormancy is clinically relevant as dormancy enhances homing and engraftment of HSCs in BM transplantation, a life-saving treatment for a variety of hematopoietic diseases. It is interesting to note that HSC proliferation impedes homing and engraftment in part due to altered membrane structures and a loss of membrane polarization (13). In fact, CD82-enriched polarized membrane domains are critical for engagement with the BM microenvironment, a first step in engraftment. Thus, CD82 expression may not only enforce dormancy but also create a physical platform necessary for engraftment. CD82 increases the molecular density of α4β1 (very late antigen or VLA-4) (14), which augments binding/adhesion to osteopontin and vascular adhesion molecule (VCAM-1) (15,16), improving transplantation efficiency. As α4β1 controls homing and mobilization (17,18), one possible explanation for reduced BM HSCs in CD82-deficient mice may be increased mobilization due to altered α4β1 clustering/expression. Altogether, regulation of cell cycle and matrix adherence by tetraspanins suggests these membrane proteins support hematopoiesis by enforcing the HSC dormancy program, and CD82 appears to play multiple roles that converge on maintaining dormant HSCs in the bone marrow.

Dormancy and self-renewal programs can be detrimental in the context of cancer, and tetraspanins have also been found to be upregulated on leukemic cells (19). CD82 is overexpressed in acute myelogenous leukemia, enhancing adhesion and possibly increasing homing and lodging in the bone marrow (20,21). CD82 expression also positively correlated with the survival of leukemic cells via the IL-10/STAT5 pathway (22) and via upregulation of antiapoptotic genes (23). Thus, in addition to enforcing dormancy, CD82 coordinates survival and homing cues. While these features are likely necessary for normal HSC function, they are exploited by leukemic stem cells, highlighting the therapeutic potential of targeting CD82 in leukemia.

The role of CD82 in HSC maintenance and function likely goes beyond an intrinsic role, and extends to the HSC niche. Hur *et al*. demonstrate an indirect impact of CD82 deficiency on the HSC microenvironment as mesenchymal stromal cells (MSCs) were found to express CD82 and exhibit additional regulation of HSC cell cycle progression. Furthermore, CD82-deficient mice exhibited increased myeloid cells and reduced B lymphocytes in the BM. The altered immune cell milieu and microenvironmental signals as a result of this distinct BM composition may additionally contribute to reduced HSC numbers and function. Myeloid-bias and a severe B lymphocyte deficit in the absence of CD82 was observed upon secondary transplant suggesting an intrinsic role for CD82 in lymphopoiesis. CD82 may also modulate important B cell survival signals, however, as CD82 associates with CD19 and major histocompatibility class I and II molecules in the plasma membrane of B lymphocytes (24,25). Thus, while CD82 may confer intrinsic differentiation and dormancy programs to HSCs, CD82 may also impact HSC function via its role in differentiated progeny and other non-hematopoietic cells, questions that will require lineage-specific knockouts to answer.

A role for DARC in HSC dormancy

CD82 activation of PKCα and ultimately TGFβ expression begged the question as to what protein may act as a ligand. Although tetraspanins are thought to primarily operate through lateral interaction within membrane domains, they do possess extracellular loops that potentially interact with neighboring cells. Prior evidence that CD82 can suppress metastases via interaction with DARC on endothelium prompted analysis of DARC in the BM. In contrast to humans, expression of DARC was not obvious in murine endothelia as assessed by flow cytometry; however, it was identified on murine BM MΦs and erythrocytes. Moreover, monocytes and MΦs were the prominent DARC$^+$ population in human cord blood. DARC on MΦs was shown to interact with CD82 both *in vitro* and through imaging analysis of murine BM where DARC$^+$ MΦs were found in contact with CD82$^+$ HSCs. To test the roles of CD82 and DARC in controlling HSC dormancy in response to stress, a single injection of 5-fluoruracil was administered. A corresponding decrease in both CD82$^+$ HSCs and DARC$^+$ MΦs was observed, culminating in HSC proliferation during recovery. Coordinated return of both CD82$^+$ LT-HSCs and DARC$^+$ MΦs, and their close proximity, suggested an instructional relationship. The authors provide evidence that DARC maintains CD82 expression on HSCs by preventing ubiquitination and degradation of CD82. In addition, recombinant human DARC (rhDARC) protein was able to maintain CD82 expression and confer protection against cell cycle entry and proliferation. Intriguingly, complete loss of CD82 could not be rescued by rhDARC, but low levels of CD82 could be increased by addition of rhDARC. This finding suggests that CD82 present in the plasma membrane is physically

stabilized by rhDARC and may serve as a dock for recycling CD82. The ability of rhDARC to preserve dormancy offers a novel therapeutic strategy for exogenous treatment of HSCs to improve HSC transplantation.

DARC is a so-called silent chemokine receptor able to bind chemokines, but not signal, thereby by acting as a sink for chemokines (26). Despite normal hematological parameters and tissue histology, mice lacking DARC are more sensitive to endotoxemia, likely owing to enhanced granulocytic infiltrates (27) and increased local concentrations of chemokines (28). DARC also sequesters chemokines for subsequent release, and evidence in humans revealed a role for DARC in regulating the circulating levels of monocyte chemoattractant protein (MCP-1) (29). It will be important to clarify any additional role for DARC in regulating HSC function *in vivo* through its ability to control the inflammatory state of the BM microenvironment.

MΦs—new members of the HSC niche club

MΦs are emerging as important regulators of HSC function and location in adult bone marrow (5,30-33). To specifically test whether DARC$^+$ MΦ depletion regulates CD82 expression, the authors administered Clodronate-loaded liposomes (Clod-lip). Clod-lip-mediated MΦdepletion was previously shown to increase HSPCs in circulation (30,31) and more recently to expand the pool of HSCs in the BM (33). Consistent with the observation that loss of CD82 initiates entry to the cell cycle and increased proliferation, McCabe *et al*. observed increased HSC proliferation 24 h post-Clod-lip administration, and, interestingly, a loss of α4β1 expression (33). The inflammatory signal G-CSF, well-known for its mobilizing properties, also reduces MΦs in the BM and increases HSC proliferation expanding the HSC pool in the BM (33,34). Therefore, the observation that MΦ depletion resulted in a striking loss of HSCs in the BM was somewhat surprising (3). One possible explanation for the difference in BM HSC numbers upon MΦ depletion between these two studies may be due to distinct routes of administration of Clod-lip. Prior publications delivered Clod-lip intravenously, whereas Hur *et al*. used the intraperitoneal route. Injection via the peritoneal cavity may elicit inflammation at the site of injection, creating a demand for myeloid cells and increased differentiation of HSPCs. Increased proliferation upon loss of contact with DARC in combination with increased differentiation may result in a loss of HSCs, accounting for the observations made by Hur *et al*. In addition, inflammatory cues act in concert to mobilize HSCs and HSPCs as was shown by the combined effect of Clod-lip-mediated MΦ depletion and G-CSF (31). The impact of the DARC-CD82 axis on mobilization of HSPCs was not reported and it is possible that the loss of CD82 expression on HSCs due to removal of DARC$^+$ MΦs results in enhanced circulating HSPCs.

A subset of MΦs have previously been linked with quiescent HSCs via expression of cyclooxygenase 2 (COX-2) (35), and DARC$^+$ MΦs appear to express the highest COX-2 demonstrating functional overlap with α-SMA$^+$ macrophages. This raises the question as to whether DARC$^+$ MΦs exert their effects on HSC via mechanisms in addition to CD82 ligation. It is also important to note that the MΦs interrogated by Hur *et al*. contain only a portion of resident MΦs, as a significant number of MΦs exist in the CD11b$^{lo/neg}$ fraction of BM (33,36). Thus, it will be important to validate the *in vitro* findings using lineage-specific DARC-deficient mice to test the specific role(s) of MΦs and other cell types, including erythrocytes, in maintaining HSC dormancy *in vivo*. The possibility of targeting MΦs for therapeutic manipulation of HSC function is exciting and warrants continued investigation.

Summary

The findings by Hur *et al*. add valuable insight to an emerging paradigm that MΦs regulate HSC dormancy and function (*Figure 1*). A number of intriguing questions remain about the CD82-DARC axis. How does the interaction between DARC and CD82 impact the bone marrow microenvironment? A dysfunctional bone marrow microenvironment can initiate myelodysplastic disease and leukemia [reviewed in (37,38)] raising important questions regarding the role(s) of MΦs in pathogenesis of leukemia. What regulates DARC expression on BM MΦs? Interferon gamma (IFNγ) maintains MΦ numbers in the BM during inflammation, but results in a loss of HSCs via enhanced proliferation (33). It is interesting to speculate that IFNγ may negatively regulate DARC expression, ultimately releasing HSCs from their quiescent state. Alternatively, IFNγ and other factors may modulate DARC-mediated chemokine binding, thus influencing the local inflammatory state. The answers to these and other questions may help facilitate therapeutic targeting of the CD82-DARC axis for improving HSC transplantation, eliminating leukemic stem cells, and manipulating HSC function *in vivo*.

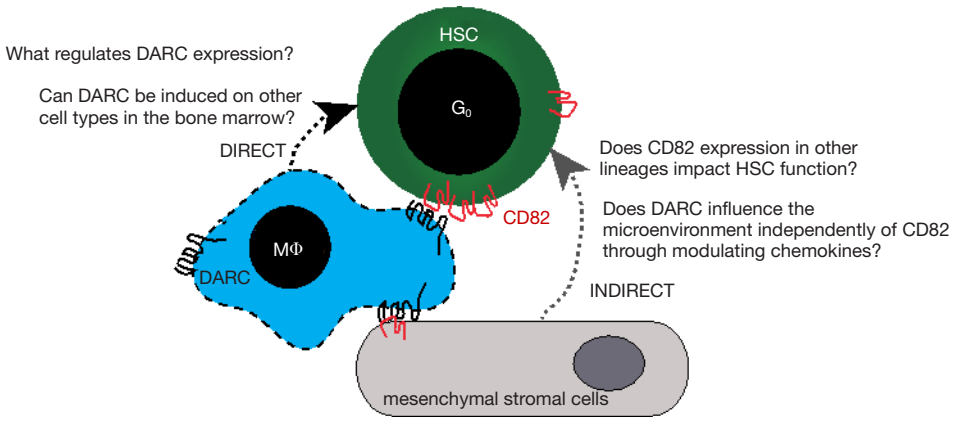

Figure 1 Overview of DARC-CD82 axis. The direct and potentially indirect mechanism(s) of action are illustrated, and several unanswered questions are highlighted.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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