Stem cell factor: the bridge between bone marrow adipocytes and hematopoietic cells

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White adipocytes serve as an energy reservoir to store excessive calories in the form of lipid droplets and protect other tissues or organs from ectopic lipid accumulation. Brown adipocytes express uncoupling protein 1 and are integral to adaptive thermogenesis. Whereas the functions of adipocytes in either white or brown adipose tissues are well documented, our knowledge of bone marrow adipocytes (BMA) remains in its infancy. Bone marrow adipose tissue (BMAT) occupies approximately 50-70% of the bone marrow volume in human adults.[1] It is a dynamic tissue and responds to multiple metabolic conditions. For example, BMAT increases with obesity, aging, diabetes, caloric restriction, and irradiation.[2] Although the significance of BMAT expansion under these conditions is still largely unknown, BMA interact locally with hematopoietic and bone cells, and contribute to global metabolism through secretion of adiponectin, leptin, stem cell factor (SCF), and other functional factors. For example, A-ZIP/F1 mice, which lack adipose tissues throughout the body, including BMAT, have delayed hematopoietic regeneration in long bones after irradiation.[3] Our latest work also observed that depletion of BMA by bariatric surgery is associated with a decrease in bone marrow erythroid cells and anemia.[4] The importance of BMA and the derived factors on hematopoiesis is further enhanced by a study in this issue of the Journal, in which Zhang *et al.*[5] demonstrate that BMAT-derived SCF mediates metabolic regulation of hematopoiesis.

Stem cell factor, also known as Kit ligand (Kitl), is a hematopoietic cytokine expressed in fibroblasts and endothelial cells, as well as in BMA.[3] Together with its receptor, c-Kit, SCF plays important roles in the maintenance of hematopoietic stem cells (HSC) and hematopoiesis. Blockade of the interaction between c-Kit and SCF with anti-c-Kit antibody promotes the clearance of HSC, which indicates the importance of Kitl/c-Kit signaling in HSC self-renewal.[6] Loss-of-function mutations in c-Kit cause macrocytic anemia, or even embryonic lethality under some severe mutations.[7] Inversely, mice with c-Kit gain-of-function mutations developed erythrocytosis compatible with myeloproliferative disorders.[8] Analyses of multiple cell populations isolated from bone marrow and adipose tissue have demonstrated that BMA and LepR-positive (+) stromal cells are the primary sources of SCF, which is required for the regeneration of HSC and hematopoiesis after irradiation.[3] Zhang *et al.* report that BMA-derived SCF is important for hematopoietic homeostasis under basal (Figure 1), obese and aging conditions, and in response to β3-adrenergic agonists.[5]

Knockout of SCF in adipocytes with an adiponectin driver does not influence circulating SCF concentrations or phenotypes of the peripheral adipose depots, which is perhaps due to compensatory expression of SCF from other sources, such as endothelial cells, fibroblasts and stromal cells. Interestingly, Zhang *et al.* observed a significant loss of SCF in the bone marrow supernatant, which indicates that BMAT is a primary source of SCF in bone marrow.[5] Deficiency of SCF in BMAT reduces the bone marrow cellularity, hematopoietic stem and progenitor cells (HSPC), common myeloid progenitors (CMP), megakaryocyte-erythrocyte progenitor (MEP) and granulocyte-monocyte progenitors (GMP) under steady-state condition. Consistent with these changes in the progenitor cells of bone marrow, mice deficient for adipocyte SCF develop macrocytic anemia and reduction of neutrophils, monocytes and lymphocytes in circulation. In contrast to results in this study, Zhou *et al.* reported that the conditional deficiency of SCF in adipocytes driven by adiponectin-Cre/ER had no effect on hematopoiesis under basal conditions.[3] Although further investigation is necessary, the discrepancy between these two studies might be due to the time-frame of SCF deletion, tamoxifen injection and/or animal lines. Of note, the deletion of SCF has no effect on the proliferation of HSPC evidenced by colony-forming assays, which suggests that defects in BMAT-derived SCF influences the bone marrow microenvironment rather than the intrinsic function of HSPC.

Since adiponectin-Cre is expressed in both peripheral adipocytes and BMA, it is possible that there might be effects on hematopoiesis that are independent of BMAT. To more specifically study effects of BMA on the bone marrow niche and hematopoiesis, Zhang *et al.* also deleted the Kitl using osterix promoter, which traces BMA but not the other adipocytes. Again, knockout of Kitl from the osterix-positive (+) cells reduced bone marrow cellularity, hematopoietic progenitor populations and mature blood cells including red blood cells (RBC), neutrophils and monocytes, which is consistent with the phenotypes from mice lacking adipocytic Kitl. Of note, in addition to BMA, osterix+ progenitors also trace to osteoblasts.[9,10] Mesenchymal and osteoblast lineage cells are involved in the maintenance and regulation of the supportive microenvironments necessary for quiescence, self-renewal and differentiation of HSC.[11,12] However, the SCF from osteoblasts is not required for HSC maintenance in adult bone marrow under steady-state conditions.[13] Although the possible effects of SCF derived from osterix+ progenitors on hematopoiesis could not be excluded and the bone phenotypes were not explored in this mouse model, it should be appreciated that authors used both adiponectin- and osterix-driven Cre enzyme to confirm the phenotypes of SCF-deficiency on hematopoiesis. These results strongly point to BMA as an important source of SCF since the common cell type traced by adiponectin and osterix drivers is the BMA; however, development of BMA-specific transgenic mouse tools will be required to truly confirm these observations of BMA and the roles of SCF in the bone marrow niche homeostasis and hematopoiesis.

The authors also investigated whether BMA-derived SCF is required for hematopoietic adaptation to aging or high fat

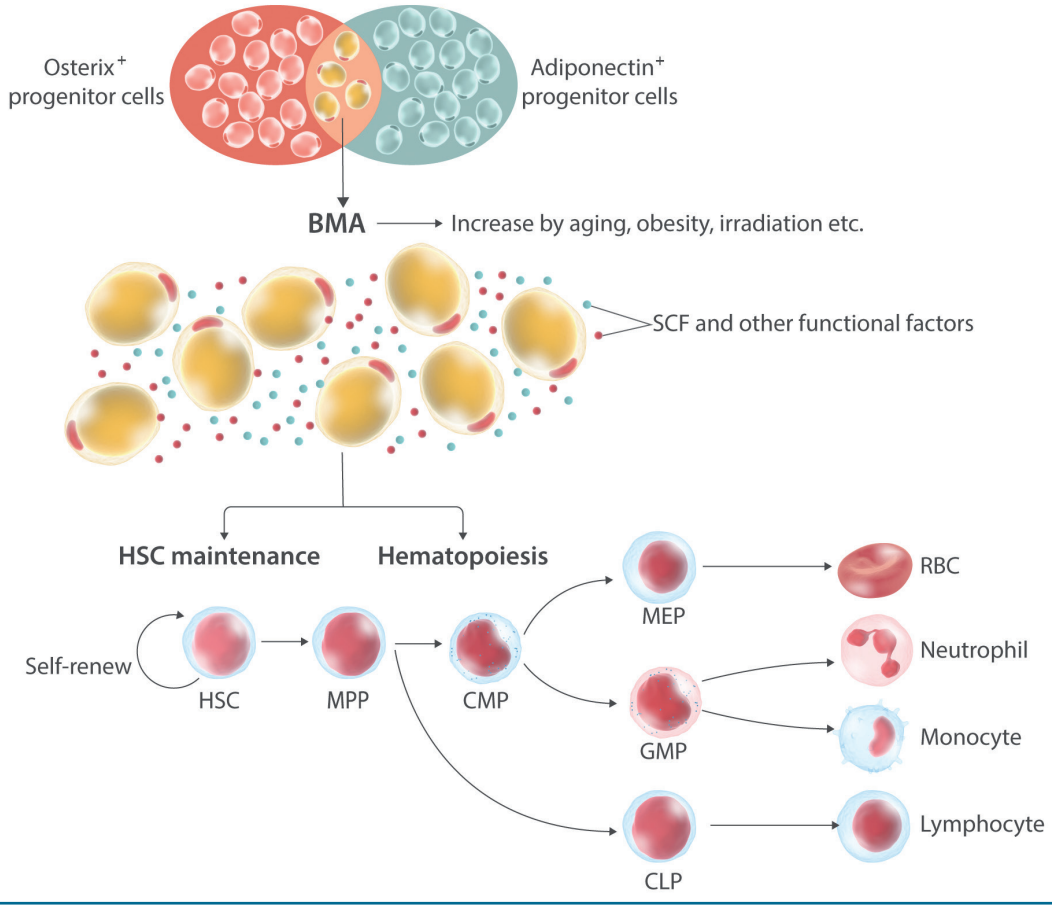

Figure 1. Bone marrow adipocytes influence the maintenance of hematopoietic stem cell (HSC) and hematopoiesis. Bone marrow cellularity is complex, but is mainly composed of hematopoietic cells and bone marrow adipocytes (BMA), which appear after birth and accumulate with age, obesity and irradiation. BMA originate from osterix-positive (+) progenitor cells and secret adiponectin, stem cell factor (SCF) and other functional factors. In this study, Zhang *et al.*[5] have demonstrated that BMAT-derived SCF plays important roles in HSC maintenance and hematopoietic differentiation under baseline, aging and obese conditions. Deficiency of SCF in BMAT hinders the self-renewal of HSC by influencing the bone marrow microenvironment and hematopoiesis through unknown mechanisms. RBC: red blood cell; MPP: multipotent progenitor; CMP: common myeloid progenitor; MEP: megakaryocyte-erythrocyte progenitor; GMP: granulocyte-monocyte progenitor; CLP: common lymphoid progenitor.

diet (HFD)-induced obesity. Whereas HFD, *per se*, did not increase the SCF concentrations in bone marrow supernatant, this treatment increased bone marrow cellularity, HSPC, and mature blood cells, including granulocytes, monocytes and lymphocytes, the effects of which were eliminated by SCF deficiency in adipocytes. Aging causes similar increases in the HSPC, especially in the myeloid lineage populations, and most of these effects required adipocyte-derived SCF. Further, these investigators explored a potential role for SCF in mediating effects of a β3-adrenergic receptor agonist. Activation of these receptors induces the lipolysis of white adipocytes, and while although BMAT lipolysis is relatively resistant to β-adrenergic signaling,[14] Zhang *et al.* observed that after administration of a β3-adrenoceptor agonist, CL316, 243, SCF expression was increased in bone marrow without significant changes in the BMA numbers.[5] Consistent with the elevated SCF in bone marrow, the numbers of HSPC, including $Lin^{-}Sca1^{+}c\text{-}Kit^{+}$ (LSK) cell, multipotent progenitor (MPP), MEP, GMP and CLP were increased by CL316, 243 injection, the effects of which were compromised by adipocyte-specific deficiency of SCF. Based on the animal models described above, it should be noted that alterations of BMAT, SCF and hematopoiesis were not tightly associated under these conditions, which suggests that hematopoietic metabolism is regulated by factors beyond BMAT and its derived SCF. The global effects of obesity, aging and β3-adrenoceptor activation cannot be excluded from this scenario. In addition, other secreted factors from BMAT may also play significant roles in hematopoiesis under these conditions. Unfortunately, the secretome of BMAT remains largely unexplored.

In summary, Zhang *et al.*[5] have extended our understanding of the roles of BMAT in the bone marrow niche and the interaction between BMA and hematopoietic cells. They thoroughly addressed their hypotheses using a variety of animal models and complete profiling of hematopoietic changes. However, due to the complexity of whole-body metabolism and the lack of BMA-specific transgenic tools, further work will be required to determine whether BMA-derived SCF regulates hematopoiesis directly through Kitl/c-Kit signaling in hematopoietic cells or indirectly by changing the microenvironment of the bone marrow niche.

Acknowledgments

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New potential players in hepcidin regulation

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The manuscript by Liu and colleagues, published in this issue of *Haematologica*, reports the identification of novel compounds able to increase hepcidin expression in normal mice as well as in animals affected by hemochromatosis and β-thalassemia intermedia (or non-transfusion-dependent thalassemia) (Figure 1A).[1]

Hepcidin is the master regulator of iron secreted from the liver and acts on ferroportin, a transmembrane protein that functions as an iron exporter.[2,3] Once hepcidin binds ferroportin, the complex is rapidly degraded, preventing iron egress.[2,3] Ferroportin is expressed in many types of cells, including enterocytes and macrophages.[2,3] Therefore, the relative abundance of hepcidin in the circulation and ferroportin on cell membranes control iron absorption (from enterocytes) and iron recycling (from macrophages).[2,3]

Hepcidin expression is regulated by iron, inflammation and erythropoiesis.[2,3] With regard to iron-mediated control of hepcidin, this is achieved through at least two mechanisms. The first senses the amount of intracellular iron in liver sinusoidal endothelial cells and responds by synthesizing BMP6, and other similar ligands, belonging to the TGFβ-like family.[2-4] Increased intracellular concentration of iron leads to secretion of BMP6 from these cells.[2-4] As a consequence, BMP6 binds and activates receptors that trigger phosphorylation of a SMAD complex and stimulate hepcidin expression in hepatic cells.[2-4]

The second mechanism senses the iron in circulation by recognizing iron-loaded transferrin molecules.[3] Molecules such as HFE, transferrin receptor-2, and others communicate intracellularly when the transferrin saturation levels increase.[3] It has been hypothesized that this sensing complex potentiates the SMAD complex activated by BMP6.[5] Alternatively, or in addition, it has been suggested that this complex acts upon hepcidin expression by decreasing the ERK1/2 pathway.[10]

Under conditions that require enhanced red cell production (as a consequence of a transient or chronic anemia), hepcidin synthesis is normally suppressed.[2] A few factors have been identified that could play a role in this mechanism, such as erythroferrone and platelet-derived growth factor BB.[7,8] In particular, erythroferrone is secreted by erythroid cells and acts as a trap ligand, limiting the activity of BMP6 and other similar molecules.[9]

Another player in the regulation of hepcidin is the molecule matriptase-2 (or TMPRSS6).[2,3] This molecule prevents hepcidin overexpression, which could lead to hypoferremia and anemia.[3,10] Although it is unclear which pathways and molecules control TMPRSS6, it has been shown that TMPRSS6 is required for erythropoietin-mediated hepcidin suppression in mice.[11,12]

In primary forms of hemochromatosis, patients show excessive iron absorption and suffer from iron overload (Figure 1A).[7,8] This happens when hepcidin, or other genes that control its expression, are mutated.[2,3] In secondary forms of hemochromatosis (as in β-thalassemia), the anemia triggers increased iron absorption, likely by increased expression of erythroferrone and other hypox-