Review

Fibrillin-1 microfibrils influence adult bone marrow hematopoiesis

Silvia Smaldone[a], **Carolina L. Bigarella**[b], **Maria del Solar**[a], **Saghi Ghaffari**[b] **and Francesco Ramirez**[a]

***a* - Department of Pharmacology and Systems Therapeutics and Institute for Systems Biomedicine,** *Icahn School of Medicine at Mount Sinai, New York, NY, 10029*
***b* - Department of Developmental and Regenerative Biology,** *Icahn School of Medicine at Mount Sinai, New York, NY, 10029*

Correspondence to Francesco Ramirez: *francesco.ramirez@mssm.edu*
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Abstract

We have recently demonstrated that fibrillin-1 assemblies regulate the fate of skeletal stem cells (aka, mesenchymal stem cells [MSCs]) by modulating TGFβ activity within the microenvironment of adult bone marrow niches. Since MSCs can also influence hematopoietic stem cell (HSC) activities, here we investigated adult hematopoiesis in mice with Cre-mediated inactivation of the fibrillin-1 (*Fbn1*) gene in the mesenchyme of the forming limbs ($Fbn1^{Prx1-/-}$ mice). Analyses of 3-month-old $Fbn1^{Prx1-/-}$ mice revealed a statistically significant increase of circulating red blood cells, which a differentiation assay correlated with augmented erythropoiesis. This finding, together with evidence of fibrillin-1 deposition in erythroblastic niches, supported the notion that this extracellular matrix protein normally restricts differentiation of erythroid progenitors. Whereas flow cytometry measurements identified a decreased HSC frequency in mutant relative to wild type mice, no appreciable differences were noted with regard to the relative abundance and differentiation potential of myeloid progenitor cells. Together these findings implied that fibrillin-1 normally promotes HSC expansion but does not influence cell lineage commitment. Since local TGFβ hyperactivity has been associated with abnormal osteogenesis in $Fbn1^{Prx1-/-}$ mice, 1-month-old mutant and wild type animals were systemically treated for 8 weeks with either a pan-TGF-β-neutralizing antibody or an antibody of the same IgG1 isotype. The distinct outcomes of these pharmacological interventions strongly suggest that fibrillin-1 differentially modulates TGFβ activity in HSC *vs.* erythroid niches.

Introduction

The adult bone marrow is the main source of bone, blood and immune cells [1,2]. As result, identification of the extrinsic regulators of stem/progenitor cell niches in the adult bone marrow is a top research priority in tissue bioengineering and skeletal and hematopoietic regenerative medicine. The adult bone marrow contains mesenchymal stem cells (MSCs; aka, skeletal stem cells) capable of forming spindle-like colonies (colony-forming unit fibroblasts, CFU-Fs) that can be expanded and differentiated into osteoblasts, chondrocytes or adipocytes under appropriate cell culture conditions [3]. MSCs also support bone marrow hematopoiesis both *in vitro* and *in vivo*, thereby linking skeletal development with the assembly of hematopoietic stem cell (HSC) niches [2–7]. In contrast to the poorly defined nature of the MSC microenvironment [3], two intertwined niches are believed to support HSC maintenance and activation (the bone niche [8,9]) and progenitor cell commitment and precursor cell differentiation (the vascular niche [10,11]). Dynamic interactions among different marrow cell types, cell-bound molecules and soluble biochemical signal have all been shown to influence the activities of stem/progenitor cells residing within specific marrow niches [1,3,12–14]. By contrast, the role of the extracellular matrix (ECM) remains largely undefined with negative implications for our ability to develop more effective, evidence-based therapeutic strategies of skeletal tissue regeneration [15].

Studies of mouse models of Marfan syndrome (MFS), a connective tissue disease caused by mutations in fibrillin-1, have demonstrated the involvement of this structural component of the architectural matrix in regulating local bioavailability of TGFβ and BMP signals that drive tissue formation, remodeling and regeneration [16]. A case in point is the skeleton where fibrillin-1 and the structurally related fibrillin-2 protein have been shown to differentially modulate TGFβ and BMP signals during bone patterning and remodeling [17]. We have recently reported that fibrillin-1 influences MSC fate determination by controlling TGFβ bioavailability within adult marrow niches [18]. Here, we investigated the role of fibrillin-1 in adult hematopoiesis in light of the functional relationship between MSC and HSC niches [2–7]. Our findings demonstrate that loss of fibrillin-1 in the mouse's marrow also causes significant hematopoietic abnormalities, such as HSC depletion and augmented erythropoiesis (polycythemia). Furthermore, the distinct outcomes of systemic TGFβ neutralization in mutant mice strongly suggest that fibrillin-1 differentially modulates TGFβ signaling within HSC and erythroid niches.

Results

Genetic inactivation of fibrillin-1 synthesis in the forming limbs of *Fbn1*$^{Prx1-/-}$ mice perturbs MSC fate determination and leads to age-dependent bone loss associated with an unusual paucity of marrow adipocytes [18]. Consistent with the darker appearance of adult limb bones (Fig. 1a), progressive expansion of the hematopoietic marrow in 3- and 6-month-old *Fbn1*$^{Prx1-/-}$ mice paralleled the age-dependent loss of trabecular bone and adipocytes recently described in these mutant mice (Fig. 1b) [18]. In light of this evidence and the functional crosstalk between MSCs and HSCs [2–7], we compared the status of hematopoiesis in 3-month-old wild type (WT) and *Fbn1*$^{Prx1-/-}$ mice. Elevated hematocrits, hemoglobin content and circulating red blood cells in mutant relative to WT mice strongly suggested dysfunctional hematopoiesis resulting in polycythemia (Fig. 1c). An *in vitro* assay designed to detect the number of colony-forming units of the erythroid lineage (CFU-E assay) implied enhanced marrow erythropoiesis (Fig. 1d). Consistent with this finding, we found that fibrillin-1 is deposited in the specialized, macrophage-containing marrow niches (aka, erythroblastic islands [19]) where erythroid progenitors proliferate and undergo terminal differentiation (Fig. 1e). Collectively, these findings support the novel notion that fibrillin-1 microfibrils normally restrict erythroid expansion.

Next, we performed flow cytometry analyses of cells flushed out from bone marrows so as to estimate the frequency of (CD34^{low}, Lin^{-}, Sca-1^{+}, c-Kit^{+} [LSK]) HSCs in 3-month-old *Fbn1*$^{Prx1-/-}$ and WT mice. The results of these analyses documented a statistically significant reduction of LSK-HSCs in MT relative to WT marrow samples (Fig. 2a). On the other hand, flow cytometry analyses showed no changes in the frequency of common myeloid progenitor (CMP), granulocyte/macrophage progenitor (GMP) and megakaryocyte/erythroid progenitor (MEP) cells (Fig. 2b). Likewise and in contrast to the results of the CFU-E assay, no appreciable differences were noted between WT and mutant marrow-derived early progenitor cells differentiated into various hematopoietic cell types (Fig. 2c). Collectively, these findings demonstrated that fibrillin-1 is a novel ECM regulator of HSC maintenance, but not myeloid cell lineage specification.

TGFβ hyperactivity in marrow niches of *Fbn1*-$^{Prx1-/-}$ mice has been implicated in a premature depletion of MSCs and osteoprogenitor cells causing age-dependent bone loss [18]. We therefore explored if a similar mechanism may account for the hematopoietic defects noted in these mutant animals. To this end, WT and *Fbn1*$^{Prx1-/-}$ mice were treated with either the pan-TGFβ-neutralizing antibody 1D11 or a control antibody of the same IgG1 isotype [18]. Treatment started at 1 month of age and ended 8 weeks later when mutant and WT mice were sacrificed to evaluate the impact of TGFβ neutralization on hematopoiesis. Similar to the beneficial effect on bone mass and trabecular microarchitecture [18], chronic administration of 1D11 prevented polycythemia in *Fbn1*$^{Prx1-/-}$ mice (Fig. 3a). By contrast, HSC frequency in 1D11- *vs.* placebo-treated mutant animals did not change even though TGFβ neutralization substantially reduced HSC abundance in WT mice (Fig. 3b). As explained in the Discussion, we interpreted these findings to indicate that fibrillin-1 differentially modulates TGFβ signals within HSC and erythroid niches.

Discussion

In spite of much research effort, our current understanding of ECM's contribution to the structural and functional requirements of bone marrow niches remains limited. Previous immunohistological analyses have localized collagen, fibronectin and laminin to discrete areas of the mouse bone marrow believed to represent functionally distinct niches [20]. Additional studies have highlighted the importance of tenascin-C and agrin in modulating HSCs and progenitor cell proliferation and survival [21,22]. The findings described here together with our previous characterization of osteopenia in MFS mice [18] are the first to identify a structural component of the ECM (fibrillin-1) that coordinates both adult osteogenesis and hematopoiesis.