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Identity of Gli1+ cells in the bone marrow

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Abstract

Bone marrow fibrosis is a critical component of primary myelofibrosis in which normal bone marrow tissue and blood-forming cells are gradually replaced with scar tissue. The specific cellular and molecular mechanisms that cause bone marrow fibrosis are not understood. A recent study by using state-of-the-art techniques including *in vivo* lineage-tracing provides evidence that Gli1+ cells are the cells responsible for fibrotic disease in the bone marrow. Strikingly, genetic depletion of Gli1+ cells rescues bone marrow failure and abolishes myelofibrosis. This work brings a new central cellular target for bone marrow fibrosis. The emerging knowledge from this research will be important for the treatment of several malignant and non-malignant disorders.

Keywords

pericytes; mesenchymal stem cells; hematopoietic stem cells; niche; microenvironment; fibrosis

Fibrosis is a pathological condition characterized by excessive production and accumulation of extracellular matrix proteins, loss of tissue architecture, and organ failure. Bone marrow fibrosis is a reactive process and a central pathological feature of primary myelofibrosis. It is characterized by the increased deposition of reticulin and collagen fibers [1]. One major problem that has confounded the field has been the lack of understanding of the biological mechanisms involved in bone marrow fibrosis [2]. The myofibroblast is acknowledged to be the key cell regulating tissue fibrosis mainly through extracellular matrix deposition [3]. Understanding what cells originate myofibroblasts is of utmost importance, since gaining control of these cells may allow us to arrest or even induce reversion of fibrosis in certain

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DISCLOSURES

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disease conditions [4]. This has been the focus of recent research with the aim to accelerate the design of targeted anti-fibrotic drugs. Several cellular populations have been implicated in fibrosis in several tissues, including circulating progenitor cells [5], endothelial cells [6], resident fibroblasts [7], epithelial cells [8], and pericytes [9]. During the last few years, a number of studies have improved our knowledge of the cellular complexity in the bone marrow [10]. Nevertheless, the biological processes underlying fibrous tissue deposition in the bone marrow are not fully understood.

Revealing the origin of myofibroblasts in the bone marrow is crucial as these cells are considered an ideal and essential target for anti-fibrotic therapy. In a recent article in *Cell Stem Cell*, Schneider and colleagues demonstrated that bone marrow Gli1+ cells differentiate into myofibroblasts in two distinct mouse models of myelofibrosis (Jak2(V617F) and thrombopoietin-induced myelofibrosis), and in bone marrow biopsies from human patients with myeloproliferative neoplasms [11] (Figure 1). The authors followed *in vivo* the fate of bone marrow Gli1+ cells by using genetic lineage-tracing technology to track specifically Gli1-expressing cells (Gli1-CreERT2/tdTomato mice). These experiments revealed that Gli1+ cells expand in the bone marrow.

Furthermore, this study quantified the contribution of Gli1+ cells to α SMA+ myofibroblasts formation; and it corresponded to approximately half of all myofibroblasts in the fibrotic bone marrow [11]. Additionally, to evaluate whether Gli1+ cells are essential to fibrosis formation in the bone marrow, Schneider and colleagues used a mouse model to specifically deplete the Gli1+ cell population, but not other cells (Gli1-CreERT2/iDTR mice). Strikingly, eliminating Gli1+ cells abolished the fibrotic phenotype and reduced osteosclerosis in the bone marrow [11]. Schneider and colleagues performed RNA-seq of bone marrow Gli1+ cells to characterize the mechanisms and potential pathways involved in the activation of these cells in bone marrow fibrosis. Particularly, they found that the chemokine CXCL4 was significantly upregulated in Gli1+ cells. Furthermore, using sophisticated co-culture experiments the authors demonstrated that hematopoietic cells-derived CXCL4 induces Gli1+ cell migration and differentiation into myofibroblasts [11].

Importantly, the authors also showed that a small molecule GANT61, an inhibitor of GLI1 protein, ameliorates myelofibrosis, by affecting the differentiation of Gli1+ cells into myofibroblasts in the murine models. Moreover, using bone marrow biopsies from patients with myelofibrosis, the authors showed that Gli1+ cells also expand in human bone marrow fibrosis and are sensitive to GANT61 inhibition [11].

This work provide a new possible central cell population to be pharmacologically targeted in bone marrow fibrosis.

PERSPECTIVES / FUTURE DIRECTIONS

Bone marrow Gli1+ cells are heterogeneous on their location within the bone marrow [11, 12]. These cells are present in the endosteal niche as well as in the perivascular niches (around sinusoids and arterioles). Thus, Gli1+ cells may correspond to distinct cell populations. Which of these subpopulations gives rise to myofibroblasts and consequently to

bone marrow fibrosis remains unanswered. Additionally, several other stromal cell types have been described in the bone marrow: NG2+, PDGFR α +, Gremlin-1+, Prx1+, LepR+, and Nestin-GFP+ cells [13]. What is the exact overlap between these cell populations with Gli1+ cells remains unknown, and whether they contribute to bone marrow fibrosis require further investigations. The roles of these stromal cellular populations in the fibrotic bone marrow as compared to physiologic conditions remains unrevealed, and should be evaluated in future studies. It will be interesting to understand how each of them and their functions are affected in the process of bone marrow fibrosis. It has been demonstrated that stromal cells within the bone marrow vary in their origin. While some stromal cells are derived from the mesoderm, others are of neural crest origin [14]. The embryonic origin and the developmental relationship of bone marrow Gli1+ subpopulations are yet to be elucidated. Do endosteal and perivascular bone marrow Gli1+ cells share a common origin? In addition to genetic cell fate mapping, transcriptomic and single cell analysis represent fundamental tools that will help us understand the roles and the origins of Gli1+ subpopulations within the bone marrow. Taking their diversity into account, Gli1+ cells will be crucial in advancing our understanding of development, disease and aging in the bone marrow.

Interestingly, the authors called Gli1+ cells mesenchymal stem cells (MSCs), based on a previous study from the same group. Kramann and colleagues demonstrated that those bone marrow Gli1+ cells express typical MSC surface markers, exhibit *in vitro* trilineage differentiation capacity, and possess colony-forming activity [12]. However, these initial three criteria for MSCs, according to the International Society for Cellular Therapy (ISCT) [15], evolved to the current thought which requires more functional *in vivo* characterization. Based on those initial criteria [15], and on the presence of cells that follow these criteria in multiple adult tissues, it was suggested that MSCs have a common origin. Due to the broad organ distribution of blood vessels, the attention had turned to pericytes as candidates to be the MSCs [16–18]. Initially, isolation of pericytes followed by long-term culture provided evidence that they acted as stem cells in various tissues [19–26]. However, it was not yet clear if pericytes behaved as stem cells *in vivo* due to the artefactual nature of many *in vitro* studies. Some clarity only came from more recent studies using genetic lineage tracing models. These studies demonstrated the capacity of pericytes to differentiate into several other cell types also *in vivo* [9, 27, 28]. Nevertheless, a recent study challenged the current view of the endogenous capacity of pericytes to differentiate into other cell types, including fibroblasts, *in vivo* [29]. Guimarães-Camboa and colleagues discovered that almost all pericytes and smooth muscle cells in several organs express the transcription factor Tbx18. Based on this knowledge, they created a mouse model in which the fate of Tbx18+ pericytes and Tbx18+ smooth muscle cells could be tracked *in vivo* (Tbx18-CreERT2/tdTomato mice). Surprisingly, Tbx18+ pericytes did not function as stem cells *in vivo* in the context of aging and in several pathological settings [29]. Thus, the discussion about pericytes' plasticity remains open [30]. In the case that the subpopulation of Gli1+ cells located in the perivascular niche corresponds to pericytes, and has the ability to differentiate into myofibroblasts, again demonstrates the *in vivo* plastic capability of pericytes. Nevertheless, whether the perivascular subpopulation of bone marrow Gli1+ cells have the ability to originate fibrosis remains unknown.

Of note, Zhou and colleagues showed that leptin receptor (LepR) is a marker for bone marrow MSCs. This group also showed that bone marrow LepR⁺ cells, which are located around both sinusoids and arterioles, are the major source of bone and adipocytes in the adult bone marrow [13]. Importantly, although Schneider et al. (2017) showed the possible lipogenic potential of Gli1⁺ cells, they did not detect leptin receptor expression in bone marrow Gli1⁺ cells [11]. Moreover, as most of Gli1⁺ cells are not perivascular, but endosteal [11, 12], this group would argue that not all bone marrow MSCs are pericytes. Flow cytometric characterization of freshly dissociated cells in future studies using Gli1CreER, Tbx18-CreER, and other genetic lineage mapping mouse models will provide an accurate clarification of the identity of MSCs in the bone marrow.

The hematopoietic stem cells (HSC) microenvironment in the bone marrow, also termed “niche”, supports the homeostasis of those cells [31]. Pro-differentiation, pro-renewal, or pro-quiescence microenvironments define the HSCs’ fate [32]. Experimental evidence has shown that deregulation of those niche regulatory mechanisms plays a key pathogenic role in a variety of hematopoietic diseases [10, 33]. Thus, understanding which cells cross-talk with HSCs in the bone marrow microenvironment is of fundamental importance. Pericytes have been defined as central components of the HSC niche [34, 35], and *in vivo* ablation of those cells in the bone marrow have detrimental effects on HSCs [34]. There are two varieties of bone marrow pericytes according to their location in the blood vessels: arteriolar and sinusoidal [36]. The majority of dormant HSCs reside in the proximity of arterioles [36]. Curiously, arterioles are preferentially located in the endosteal part of the bone marrow. Although, the majority of Gli1⁺ cells are located in the endosteal niche, depletion of Gli1⁺ cells using Gli1CreER/iDTR mice did not reduce the numbers of HSCs in the bone marrow [11], indicating that Gli1⁺ cells are distinct from pericyte populations previously described important for maintenance of HSCs in the bone marrow [36–38]. Interestingly, if endosteal Gli1⁺ cells correspond to cells of the osteoblastic lineage as suggested in this study, these findings further support recent works demonstrating that osteoblasts are not essential niche cells for HSCs [39]. Nevertheless, Schneider and colleagues did not observe changes in lymphoid cells after Gli1⁺ cells depletion, while other groups show that ablation of cells of the osteoblastic lineage leads to loss of lymphoid progenitors in the bone marrow [39]. Future studies should address the reasons for these discrepancies. These differences may be due to existence of Gli1[−] osteoblasts important for the maintenance of lymphoid progenitors in the bone marrow.

Bone marrow fibrosis is seen in a variety of non-malignant and malignant disease states. It is a complication of several has also been described in association with non-malignant pathologies such as chronic autoimmune diseases (systemic lupus erythematosus), infectious syndromes, rickets due to vitamin D deficiency, and after exposure to radiation or toxins. It has also been described in association with hematological neoplasms such as myelodysplastic syndromes, myeloproliferative disorders, systemic mastocytosis, lymphomas, lymphoid and myeloid acute leukemias, and medullar cancer metastasis. [2]. Whether bone marrow Gli1⁺ cells are the main source of fibrosis in all these non-malignant as well as malignant conditions still needs to be addressed. Interestingly, as only 50% of myofibroblasts in myelofibrosis were derived from the Gli1 lineage [11], it remains to be revealed which are the other sources of these cells in the bone marrow; and whether these

sources vary between different pathologies. In a recent study, using mice transplanted with myelofibrosis patient-derived bone marrow cells, Verstovsek and colleagues showed that fibrosis may also be caused by monocyte-derived fibrocytes [40]. Future experiments will likely shed more light on the exact contribution to myofibroblasts accumulation from distinct sources. Importantly, a distinction should be made between two types of fibrocytes in myelofibrosis: malignant (neoplastic fibrocytes) and reactive (fibrocytes differentiated from normal monocytes). This may be addressed by the induction of myelofibrosis in CD11b-Cre/TdTomato mice in which monocyte-derived cells will be labeled.

CLINICAL INTERVENTION FOR MYELOFIBROSIS

Currently, allogeneic hematopoietic stem cell transplant is the only potentially curative treatment for myelofibrosis [41]. Myelofibrosis can affect a wide range of ages, although is more common in elderly patients. Thus, very few patients are eligible for transplant due to the high risk from toxic conditioning chemotherapy and several complications after the bone marrow transplantation, such as graft failure, infections, and graft-versus-host disease [42]. Patients who can not receive stem cell transplants require other therapies to manage myelofibrosis symptoms [43]. Unfortunately, the conventional drug therapies do not provide survival benefits to patients with myelofibrosis or change the natural course of disease progression. Additionally, they offer only modest response rates to myelofibrosis-related symptoms [44].

Schneider and colleagues propose the use GANT61, an experimental agent in preclinical studies, to eliminate Gli1-expressing cells in myelofibrosis [11]. Nonetheless, GANT61 blocks both GLI1- and GLI2- mediated transcription [45]. Differences in the biological activities of GLI1 and GLI2 are evident, since GLI1 knockout mice have no obvious phenotype [46], in contrast to GLI2 knockout mice which die at birth [47, 48]. Gli2 is important for several physiological functions [49], and possibly is expressed in a different subset of bone marrow cells than Gli1. Consequently, blocking Gli2 may cause several undesired side effects in the patients with myelofibrosis. Thus, the aim of future studies should be to develop a more specific drug that will target only Gli1+ cells. Myelofibrosis is characterized by several systemic effects. As Gli1+ cells are present in several other organs beside the bone marrow, the effects of the blocking or depletion of Gli1+ cells in other organs also need to be taken into consideration when developing a specific targeted therapy.

Although Gli1+ cells play a central role in bone marrow fibrosis, it remains unknown what is the role of those cells in physiologic conditions. From a drug development perspective, Gli1+ cells provide a central cellular target with a stereotyped molecular repertoire and responses to signals. Nevertheless, it may be challenging to limit deleterious effects of Gli1-inhibition, while preserving the healthy ones. Thus, developing a drug that will be able to arrest solely the Gli1+ fibrosis-producing cells in the bone marrow, will be the aim of future studies in myelofibrosis research.

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Figure 1. Gli1+ cells take center stage in bone marrow fibrosis

It is well accepted that the bone marrow hosts various cells with distinct functions in its microenvironment. Gli1+ cells are present around the endosteum and the blood vessels (arterioles and sinusoids). The study of Schneider and colleagues now reveals that bone marrow Gli1+ cells generate fibrosis-producing cells in myelofibrosis [11]. Cxcl4 induces Gli1+ cells migration and transdifferentiation into myofibroblasts. Using Gli proteins as a target with GANT61 holds promise for the treatment of this disorder. Thus, from a drug development perspective, Gli1+ cells provide a central cellular target for bone marrow fibrosis.