

Myeloproliferative neoplasms - Section 15

Fibrosis driving myofibroblast precursors in MPN and new therapeutic pathways

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Take home messages

- Myeloproliferative neoplasm disorders originate from acquired mutations in HSCs and establish a proinflammatory and fibrogenic niche environment that disrupts normal hematopoiesis and stromal cell populations.
- Gli1⁺ and LepR⁺ stromal cells have been recently described as being fibrosis-driving cells that differentiate into myofibroblasts and produce extracellular matrix in the bone marrow.
- While advances have been made in developing specific pathway inhibitors; further studies are investigating the use of combinational therapies to better treat these complex malignancies.

Introduction

Myeloproliferative neoplasm (MPN) is a group of diseases that are closely associated with bone marrow (BM) fibrosis, which is the continuous replacement of blood-forming cells with excessive scar tissue. In particular, primary myelofibrosis (PMF) is the prototypical example of the progressive development of BM fibrosis and BM failure. Despite our advanced understanding of mutations occurring in hematopoietic stem cells in MPN, the specific mechanisms that cause BM fibrosis in PMF are not understood, in particular as the cells driving fibrosis have remained obscure for many years and are far less understood than in organ fibrosis. Overall, the microenvironment becomes more inflammatory and the presence of cytokines and chemokines promotes fibrosis and the dysregulation of normal hematopoietic stem cells (HSCs). Importantly, accumulative data suggest that BM fibrosis in MPN is not simply a disease of the mutated HSC, but rather a disease of the entire HSC niche.

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This paradigm has important consequences for the development of novel targeted therapeutics aimed at targeting both the malignant hematopoietic clone but also the progressing fibrotic transformation.

The only intervention thought to be curative in myelofibrosis is allogeneic hematopoietic stem cell transplantation, but the high treatment-related morbidity and mortality limit its use to intermediate- or high-risk patients deemed fit to undergo the procedure. Hence, the majority of PMF patients are treated with palliative therapies designed to alleviate symptoms and improve quality of life. The majority of MF patients carry a JAK2 gain-of-function mutation, known as JAK2 V617F, which leads to the constitutive activation of the JAK/STAT pathway.^{1,2} However, JAK inhibitors such as ruxolitinib provide only limited reduction in allelic burden, and instead appear to block inflammatory cytokine activity rather than stem-cell derived clonal proliferation, which primarily drives the disorder.³ Hence, there is a strong clinical need for specific treatments that directly target the cause of the driver mutation or specific pathway candidates that drive fibrosis-causing cells.

Current state of the art

Recent genetic fate tracing studies have shown that Gli1⁺ and LepR⁺ stromal cells are progenitors of myofibroblasts in BM fibrosis,^{4,5} which are generally accepted to be the fibrosis-causing cells across multiple organ systems. In murine models of BM fibrosis/PMF, Gli1⁺ cells are recruited from the endosteal niche, become activated and differentiate into myofibroblasts (Fig. 1). Importantly, in murine models of thrombopoietin-induced fibrosis, genetic ablation of these Gli1⁺ stromal cells completely ameliorated BM fibrosis and prevented BM failure.^{4,5}

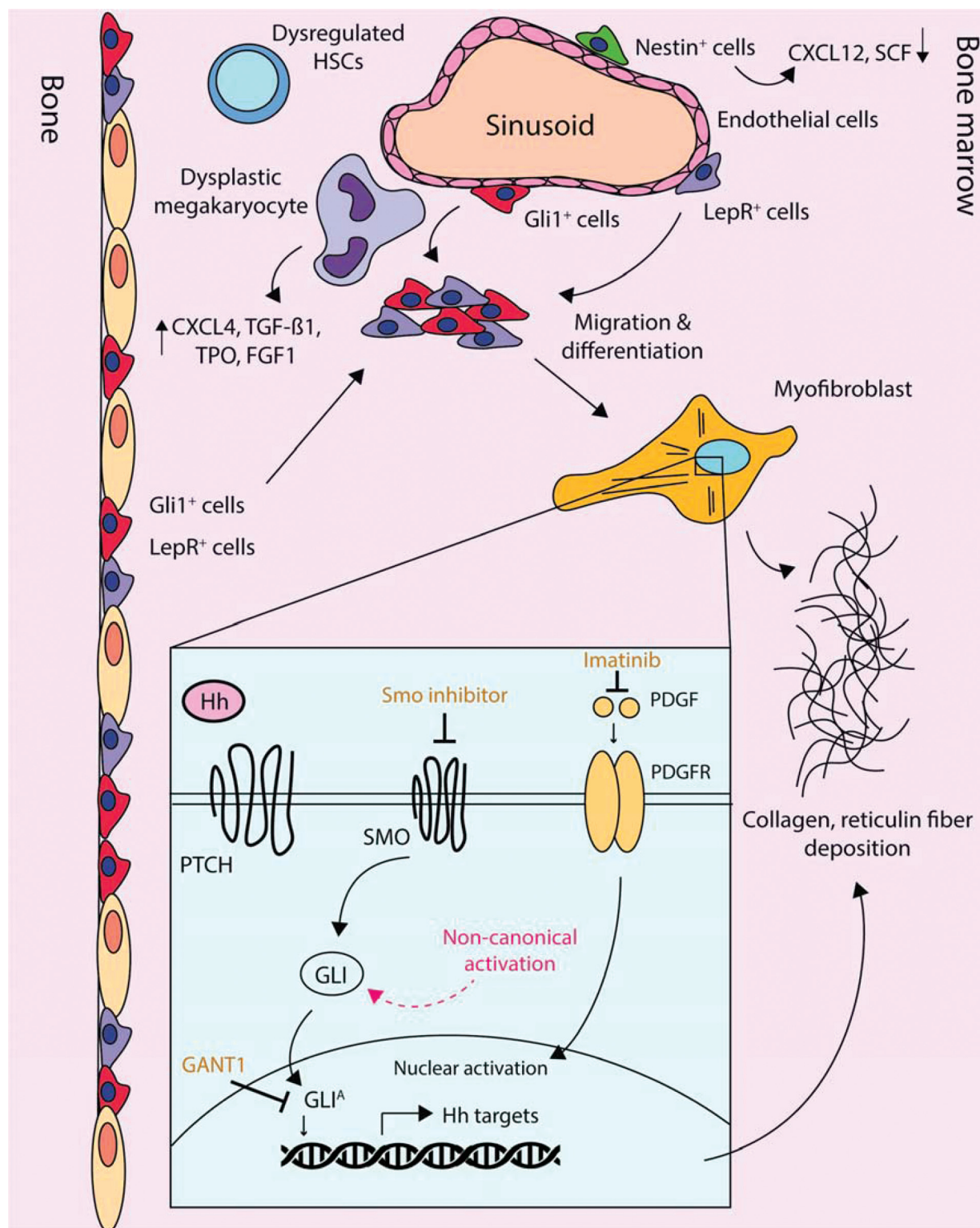


Figure 1. Reprogrammed bone marrow niche with specific focus on recently identified stromal cell populations relevant to MPN. MPN clone gives rise to mutant, dysplastic megakaryocytes that secrete CXCL4 and other fibrogenic cytokines. This release of proinflammatory molecules alters the sensitive bone marrow microenvironment, which affects stromal populations such as Nestin⁺ cells that lose their capacity to support hematopoiesis (CXCL12, SCF). Through these changes, other stromal populations such as Gli1⁺ and LepR⁺ acquire an abnormal phenotype, leading to activation, increased proliferation, and migration into the marrow space where they differentiate into myofibroblasts and deposit collagen and reticulin fibers. Canonical Hedgehog signaling can be inhibited by various smoothened (SMO) inhibitors, although recent data suggest that Gli can be directly inhibited by GANT1 or through inhibiting the noncanonical pathway. Other pathways, such as PDGF signaling, are also involved in fibrotic transformation, and can be targeted by specific inhibitors such as Imatinib. It was shown that PDGF may activate Gli through noncanonical signaling. MPN = myeloproliferative neoplasm.

RNA-sequencing experiments in mouse models of PMF indicated that megakaryocyte-associated genes were significantly up-regulated, and led to the identification of CXCL4 as a potential activator of Gli1⁺ cells. Furthermore, it was shown that the expression of Hedgehog (Hh) target genes is significantly increased in patient granulocytes, and that Gli1 expression is significantly increased in MPN patients.^{*6} However, the exact role and contribution of the Hh pathway in MF are still not completely understood. GANT1, a small molecule Gli protein inhibitor, is able to inhibit Gli1⁺ cell expansion and myofibroblast differentiation, and attenuates fibrosis severity, in both murine and human Gli1⁺ MSCs.^{*5} Therefore, there is strong evidence for targeted therapies of Gli proteins in BM fibrosis, either as monotherapy or combined therapy with other agents. In particular, the combination of JAK2 inhibitors with inhibitors of the Hh pathway might provide a novel therapeutic avenue to target clonal myeloproliferation and fibrosis. However, thus far, only smoothened (SMO) inhibitors, which inhibit canonical (Hh ligand dependent) signaling, have been tested with varying success.⁷ This is likely because Gli protein in Gli1⁺ cells can also be activated via noncanonical Hh signaling (ie, by phosphoinositide-3-kinase-AKT, TGF β , PDGF signaling), explaining the variable response of SMO inhibition in MF patients.

Another population of stromal cells shown to directly contribute to BM fibrosis through their differentiation into myofibroblasts are LepR⁺ cells.^{*4} During normal BM function, LepR⁺ stromal cells produce CXCL12, an important support factor that is crucial for HSC survival and regulation.⁸ In BM fibrosis, these cells have been shown to expand significantly and differentiate upon activation of the PDGFR α pathway, a pathway that is well known to be important in organ fibrosis (Fig. 1). Administration of Imatinib, which blocks the activity of several tyrosine kinases, including PDGFR α , or conditional deletion of platelet-derived growth factor receptor α (*Pdgfra*) from LepR⁺ stromal cells, ameliorated BM fibrosis, indicating that the PDGFR α pathway plays an important role in fibrosis not only in solid organs but also in BM fibrosis.^{*4}

While not directly involved in the deposition of fibrosis, Nestin⁺ stromal cells are also affected by the proinflammatory environment during PMF.^{*9} In the presence of an MPN hematopoietic clone, Nestin⁺ stromal cells undergo apoptosis and alter their activity, shown by a down-regulation of hematopoiesis support cytokines such as CXCL12 and SCF, thereby altering the BM niche (Fig. 1). Nestin⁺ cells are innervated by sympathetic nerve fibers and regulate normal HSCs. In MPN, IL-1 β produced from mutant HSCs results in neural damage that depletes Schwann cells leading to neuropathy and accelerated MPN progression.^{*9} Overall, these studies highlight the idea of a self-reinforcing malignant niche, which favors disease progression instead of normal HSCs by means of aberrant cytokine production and dysregulated differentiation programs.

Future perspectives

Other strategies aiming at preventing or slowing down the development of the profibrotic niche could prove useful in restoring normal hematopoiesis and disrupting the malignant self-reinforcing niche. Several immunomodulatory drugs such as thalidomide and lenalidomide, proteasome inhibitors (bortezomib), and VEGF-targeting agents (sunitinib and bevacizumab) have been tested to modify the microenvironment in patient subsets, with mixed results hampered by tolerability issues.¹⁰ Inflammatory and fibrogenic cytokines that are known to

partially drive fibrotic transformation and BM niche remodeling in mice, such as PDGF and TGF- β , can also likely be targeted using suppressing, neutralizing, or antagonizing antibodies currently available in the clinic, or in combination with ruxolitinib, an already active area of research in PMF.¹¹ The antifibrotic compound Pirfenidone (FDA approved for idiopathic pulmonary fibrosis), which inhibits TGF- β and p38 signaling, was trialed in 2001 but led to no improvement in anemia, spleen weight, or BM fibrosis. Subsequently, other strategies such as the biologic simtuzumab may focus on inhibiting extracellular matrix deposition, by interfering with the cross-linking of collagen and elastin fibers, although this showed limited effect on BM fibrosis severity.¹² Hypomethylating agents such as decitabine were shown to increase overall survival in high-risk PMF patients,¹³ although additional treatment modalities combining decitabine and other active agents are required for improved income. With our understanding of BM fibrosis and its dysregulated pathways, it seems that targeting inflammatory or fibrogenic cytokines alone may not be enough, and instead requires the targeting of multiple pathways to effectively block the interaction between malignant hematopoietic cells, inflammatory cytokines, dysplastic megakaryocytes, and fibrosis-driving cells.

Conclusion

The recent identification of Gli1⁺ and LepR⁺ cells as myelofibrotic precursors is the first important step in developing antifibrotic therapies for PMF, together with the identification of deregulated pathways that provide attractive therapeutic avenues.

References

- Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet*. 2005;365:1054–1061.
- Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005;352:1779–1790.
- Tefferi A. JAK inhibitors for myeloproliferative neoplasms: clarifying facts from myths. *Blood*. 2012;119:2721–2730.
- Decker M, Martinez-Morente L, Wang G, et al. Leptin-receptor-expressing bone marrow stromal cells are myofibroblasts in primary myelofibrosis. *Nat Cell Biol*. 2017;19:677–688.
- This study identifies LepR⁺ cells as one of the fibrosis-driving cell populations.
- Schneider RK, Mullally A, Dugourd A, et al. Gli1⁺ mesenchymal stromal cells are a key driver of bone marrow fibrosis and an important cellular therapeutic target. *Cell Stem Cell*. 2017;20:785–800.
- This study identifies Gli1⁺ cells as one of the populations responsible for fibrosis formation in the bone marrow.
- Bhagwat N, Keller MD, Rampal RK, et al. Improved efficacy of combination of JAK2 and Hedgehog inhibitors in myelofibrosis. *Blood*. 2013;122:666.
- This primary study investigates one of the first combination therapies involving JAK2 inhibitors and Hedgehog inhibitors, with overall positive results.
- Sasaki K, Gotlib JR, Mesa RA, et al. Phase II evaluation of IPI-926, an oral Hedgehog inhibitor, in patients with myelofibrosis. *Leuk Lymphoma*. 2015;56:2092–2097.
- Ding L, Saunders TL, Enikolopov G, et al. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature*. 2012;481:457–462.
- Arranz L, Sánchez-Aguilera A, Martín-Pérez D, et al. Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms. *Nature*. 2014;512:78–80.

This study identifies the importance of sympathetic nerve regulation and niche damage in the development of MPNs in bone marrow.

10. Rambaldi A, Barbui T, Barosi G. From palliation to epigenetic therapy in myelofibrosis. *Hematol Am Soc Hematol Educ Program*. 2008;2008:83–91.
11. Kramann R, Schneider RK. The identification of fibrosis-driving myofibroblast precursors reveals new therapeutic avenues in myelofibrosis. *Blood*. 2018;131:2111–2119.
12. Verstovsek S, Savona MR, Mesa RA, et al. A phase 2 study of simtuzumab in patients with primary, post-polycythaemia vera or post-essential thrombocythaemia myelofibrosis. *Brit J Haematol*. 2017;176:939–949.
13. Badar T, Kantarjian HM, Ravandi F, et al. Therapeutic benefit of decitabine, a hypomethylating agent, in patients with high-risk primary myelofibrosis and myeloproliferative neoplasm in accelerated or blastic/acute myeloid leukemia phase. *Leuk Res*. 2015;39:950–956.