tumours: a retrospective cohort study. Lancet. 2012;380(9840):499-505.

8. Fernandez-Antoran D, Piedrafita G, Murai K, et al. Outcompeting p53-Mutant Cells in the Normal Esophagus by Redox Manipulation. Cell Stem Cell. 2019;25(3):329-341.e6.

9. Rodrigues-Moreira S, Moreno SG, Ghinatti G, et al. Low-Dose Irradiation Promotes Persistent Oxidative Stress and Decreases Self-Renewal in Hematopoietic Stem Cells. Cell Rep. 2017;20(13):3199-3211.

10. Henry E, Souissi-Sahraoui I, Deynoux M, et al. Human hematopoietic stem/progenitor cells display ROS-dependent long-term hematopoietic defects after exposure to low dose of ionizing radiations. Haematologica. 2020;105(8):2044-2055.

11. Ito K, Hirao A, Arai F, et al. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat Med. 2006;12(4):446-451.

12. Miyamoto K, Araki KY, Naka K, et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell. 2007;1(1):101-112.

13. Tothova Z, Kollipara R, Huntly BJ, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress.

Cell. 2007;128(2):325-339.

14. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. Cell. 2005;120(4):483-495.

15. Nissanka N, Moraes CT. Mitochondrial DNA damage and reactive oxygen species in neurodegenerative disease. FEBS Lett. 2018;592(5):728-742.

16. Kawamura K, Qi F, Kobayashi J. Potential relationship between the biological effects of low-dose irradiation and mitochondrial ROS production. J Radiat Res. 2018;59(suppl_2):ii91-ii97.

17. Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. Science. 2012;337(6098):1062-1065.

18. Ho TT, Warr MR, Adelman ER, et al. Autophagy maintains the metabolism and function of young and old stem cells. Nature. 2017;543(7644):205-210.

19. Ito K, Turcotte R, Cui J, et al. Self-renewal of a purified Tie2+ hematopoietic stem cell population relies on mitochondrial clearance. Science. 2016;354(6316):1156-1160.

20. de Almeida MJ, Luchsinger LL, Corrigan DJ, Williams LJ, Snoeck HW. Dye-Independent Methods Reveal Elevated Mitochondrial Mass in Hematopoietic Stem Cells. Cell Stem Cell. 2017;21(6):725-729.e4.

Busy signal: platelet-derived growth factor activation in myelofibrosis

Anna E. Marneth[1] and Ann Mullally[1,2,3]

[1]Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston; [2]Dana-Farber Cancer Institute, Harvard Medical School, Boston and [3]Broad Institute, Cambridge, MA, USA.

E-mail: ANN MULLALLY - amullally@partners.org

The pathogenesis of myelofibrosis, a bone marrow (BM) disorder characterized by megakaryocytic hyperplasia and the deposition of extracellular matrix components such as reticulin, remains incompletely understood.

Using a mouse model of myelofibrosis (i.e. Gata-1^{low} mice), Kramer et al.[1] sought to identify key signaling molecules that play a role in early myelofibrosis development. GATA-1 is a transcription factor that is key to megakaryocyte development, and its downregulation results in expansion and abnormal maturation of megakaryocytes.[2] Importantly, low GATA-1 expression has been demonstrated in patients with myelofibrosis,[3] and GATA-1 mutations are found in megakaryocytic leukemias.[2]

New key findings

Unlike several widely used myelofibrosis mouse models that rely on BM transplantation to engender fibrosis, primary Gata-1^{low} mice gradually develop myelofibrosis spontaneously.[4] Due to its slow progression, this model allows for analysis at prefibrotic (5 months), early fibrotic (10 months), and overtly fibrotic (15 months) stages. A strength of the study by Kramer et al. is the application of an unbiased approach (i.e. RNA sequencing) to interrogate the changes that occur in receptor tyrosine kinase pathways during the development of myelofibrosis. Using bulk RNA sequencing on unfractionated BM (including stromal cells), the authors identified the platelet-derived growth factor (PDGF) pathway as significantly up-regulated in early fibrotic Gata-1^{low} mice compared to wild-type mice. Additionally, the authors analyzed protein expression of PDGF receptors and ligands on BM sections at the three aforementioned time points; this allowed them to study the PDGF pathway in a spatio-temporal manner.

In addition to demonstrating increased transcript expression of PDGF receptor a (Pdgfra) and Pdgfrb, as well as the ligand Pdgfb, in fibrotic Gata-1^{low} mice, the authors employed a novel technique called in situ proximity ligation assay to determine protein localization. They found that the receptor PDGFRβ and ligand PDGF-B are in close proximity in the setting of overtly fibrotic BM, suggesting binding of the ligand to the receptor and increased PDGF-B signaling. Furthermore, their data suggest that the most important cell types involved in PDGF signaling are megakaryocytes, which express PDGFRα and secrete the ligand PDGF-B, and spindle-shaped stromal cells which express PDGFRβ (Figure 1).

Despite these findings, Kramer et al. did not detect increased PDGFRβ tyrosine phosphorylation, a marker of receptor activation. They suggest that the phosphatase TC-PTP (PTPN2) may play a role in dephosphorylation of PDGFRβ and show that TC-PTP is in close proximity to PDGFRβ in fibrotic Gata-1^{low} mice. There are two main potential explanations for these findings. Either: (i) PDGF receptor activation is transient and rapidly down-regulated; or (ii) PDGF receptor activation is rapidly reset by phosphatases such as TC-PTP after ligand binding. Rapid downregulation would call into question the importance of the PDGF pathway in myelofibrosis, while a rapid reset may increase signaling in the presence of ligand and potentially contribute to the development of myelofibrosis. Further investigation of PDGF signaling in human myelofibrosis will be required to fully resolve this question.
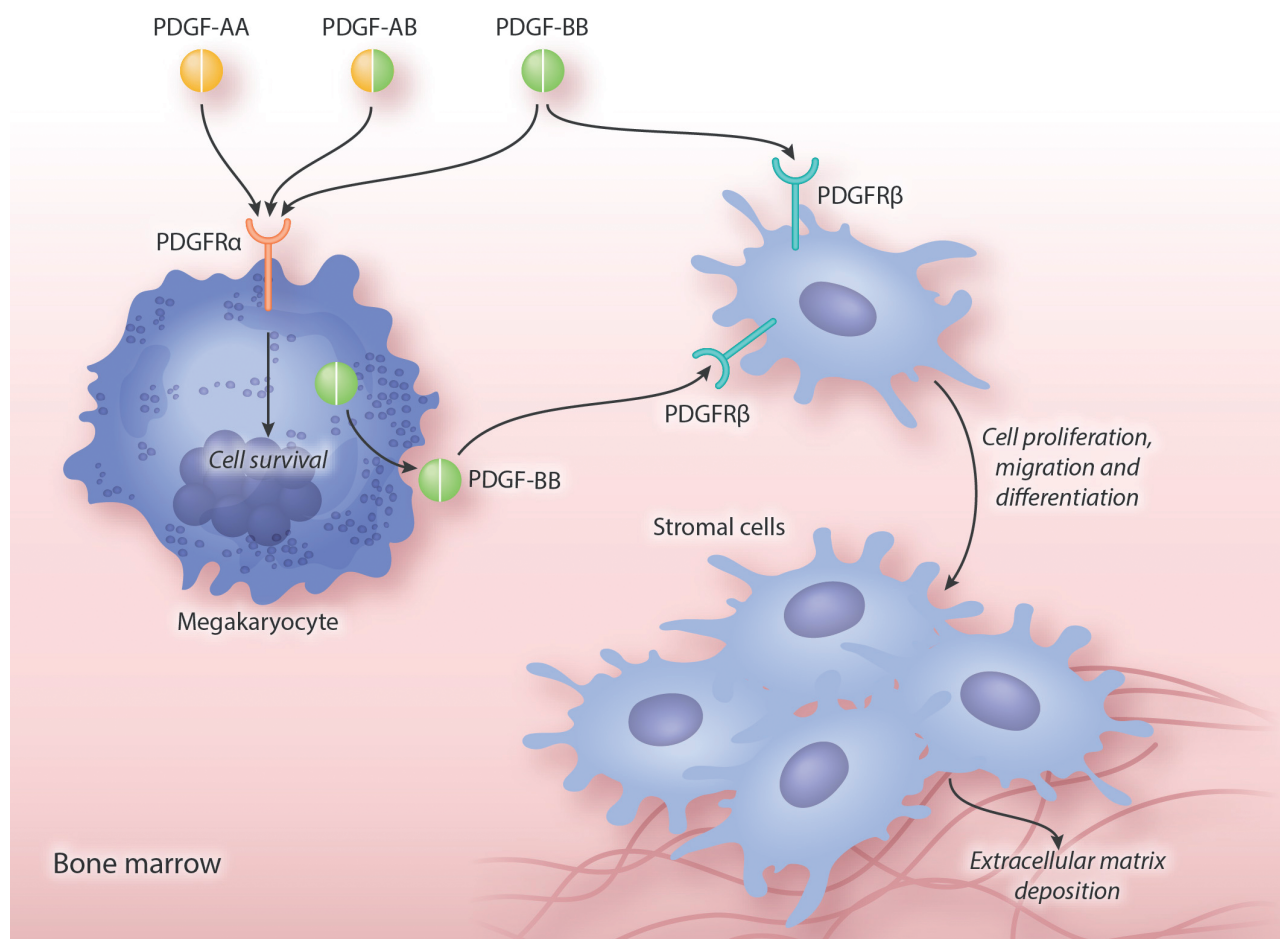
Figure 1. Platelet-derived growth factor (PDGF) signaling in myelofibrosis. Previous (human) data and the study by Kramer *et al.*[1] indicate that the ligands PDGF-A and PDGF-B and their receptors PDGFRα and PDGFRβ play important roles in myelofibrosis development. PDGFRα is mainly expressed on megakaryocytes and can be activated by the dimeric ligands PDGF-AA, PDGF-AB, and PDGF-BB. PDGFRβ is expressed on stromal cells and can be activated through the ligand PDGF-BB. PDGF-BB stimulates stromal cell proliferation, migration and differentiation, which in turn causes extracellular matrix deposition and myelofibrosis development.

Platelet-derived growth factor pathway as a potential biomarker for myelofibrosis development

Platelet-derived growth factors are growth factors for fibroblasts and stromal cells. Importantly, upregulation of the receptors PDGFRα in megakaryocytes and PDGFRβ in stromal cells, as well as upregulation of their ligands PDGF-A and PDGF-B, has been shown in established human myelofibrosis.[5-7] Moreover, the grade of myelofibrosis in myeloid malignancies correlates with the level of PDGFRβ expression in activated fibroblasts.[6] These data suggest that PDGF signaling contributes to myelofibrosis development.

Myelofibrosis occurs in the context of megakaryocyte disorders, encompassing both inherited bleeding and platelet disorders[8,9] and myeloid malignancies, most commonly, myeloproliferative neoplasms (MPN). Although patients with MPN can present with *de novo* myelofibrosis (i.e. primary myelofibrosis, PMF), it can also occur as a complication of antecedent MPN (i.e. post-polycythemia myelofibrosis, PPV-MF or post-essential thrombocythemia myelofibrosis, PET-MF). In patients with PV and ET, it is currently difficult to predict who will progress to myelofibrosis. The study by Kramer *et al.* raises the ques-

tion of whether increased PDGF signaling could serve as an early biomarker for myelofibrosis development.

Bedekocivs *et al.*[6] previously assessed PDGFRβ expression in fibrotic and non-fibrotic BM from several myeloid malignancies and proposed that elevated PDGFRβ expression could indicate a prefibrotic state. Kramer *et al.* found increased PDGFRα expression in the prefibrotic stage, but no increase in PDGFRβ or the ligands PDGF-A and -B. In future studies, it would be informative to measure the dynamics of PDGF components in human myelofibrosis development, using a longitudinal study to determine their predictive and prognostic value.

Targeting the platelet-derived growth factor pathway

In conjunction with prior (human) studies, this study by Kramer *et al.* suggests that the PDGF pathway is a potential therapeutic target in myelofibrosis. PDGFR are one of the main targets of the tyrosine kinase inhibitor, imatinib.[10] Treatment with imatinib has demonstrated clinical benefit in patients with hypereosinophilic syndromes and chronic myeloproliferative disorders who have chromosomal translocations involving PDGFRα and PDGFRβ, respectively.[11,12] Thrombopoietin (Thpo) is the major stimulant for

megakaryopoiesis and its overexpression engenders myelofibrosis in mouse models.[13] Using a Thpo overexpression model, Decker et al.[14] showed that stromal deletion of PDGFRα or treatment with imatinib suppressed stromal cell expansion and ameliorated myelofibrosis. In addition to imatinib, other methods of targeting the PDGF pathway are currently under investigation in different fibrosis models, such as PDGF/PDGFR-blocking antibodies and aptamers.[15]

Dual targeting of JAK and JAK/platelet-derived growth factor downstream pathways

Primary myelofibrosis is caused by MPN phenotypic driver mutations (i.e. in *JAK2, CALR* or *MPL*) that result in constitutive activation of JAK-STAT signaling.[16] Although JAK2 inhibitors such as ruxolitinib reduce constitutional symptoms and splenomegaly, and may stabilize myelofibrosis, they do not have substantial disease-modifying activity in MPN. Inhibiting other tyrosine kinases including PDGF receptors is not sufficient either, since imatinib treatment in PMF was disappointing.[17] A combinatorial approach involving JAK2 and PDGF inhibition in MPN could be considered, although hematologic toxicity is a real concern.[18]

An alternative treatment strategy involves simultaneously inhibiting JAK-STAT and MEK-ERK signaling. A recent MPN preclinical study showed that JAK2 inhibitors induce a strong reduction in STAT signaling but only marginally reduce MEK/ERK signaling.[19] Multiplexed analyses of 34 secreted factors in *Jak2* V617F-mutant mice showed that transcript levels of the receptor Pdgfra, as well as the ligands Pdgfa and Pdgfb, were maintained in BM and spleen during ruxolitinib treatment.[19] Additional experiments showed that PDGF signaling through MEK/ERK was not reduced upon ruxolitinib treatment. Combined treatment with JAK2 and MEK inhibitors was superior over inhibition with either compound alone in mouse models of *Jak2* V617F and MPLW515-induced myelofibrosis, and reduced Pdgfra, Pdgfa, and Pdgfb transcript expression. These data suggest that combined MEK/JAK2 inhibition may be efficacious in treating MPN.

Conclusions and future directions

In conclusion, Kramer *et al.* have methodically and elegantly analyzed the sequential changes that occur in the BM during the initiation and progression of myelofibrosis in Gata1^{low} mice and identified upregulation of the PDGF pathway as a hallmark of myelofibrosis. Their work suggests that increased PDGFR expression could be used as an early biomarker for myelofibrosis development. Given the paucity of reliable myelofibrosis biomarkers, this finding warrants further study in MPN patients. Additionally, now that next generation sequencing platforms are increasingly used to identify genetic predictors of progression to myelofibrosis in MPN, it would be interesting to study whether increased PDGF expression correlates with certain genetic subsets of MPN. Finally, given recent advancements enabling combined single-cell mutational and transcriptomic analyses,[20] it will be possible to determine precisely which cellular sub-populations in the BM (both hematopoietic and stromal) are involved in PDGF signaling early in the course of myelofibrosis. Since a multitude of profibrotic factors are up-regulated in myelofibrosis, the therapeutic

efficacy of inhibiting a single pathway, especially in advanced disease, may be limited. However, the identification and early targeting of pathways that are activated during the initial stages of myelofibrosis may prove more fruitful.

References

1. Kramer F, Dernedde J, Mezheyeuskiet A, et al. Platelet-derived growth factor receptor β activation and regulation in murine myelofibrosis. Haematologica 2020;105(8):2083-2094.
2. Shimizu R, Engel JD, Yamamoto M. GATA1-related leukaemias. Nat Rev Cancer. 2008; 8(4):279-287.
3. Vannucchi AM, Pancrazzi A, Guglielmelli P, et al. Abnormalities of GATA-1 in megakaryocytes from patients with idiopathic myelofibrosis. Am J Pathol. 2005;167(3):849-858.
4. Vannucchi AM, Bianchi L, Cellai C, et al. Development of myelofibrosis in mice genetically impaired for GATA-1 expression (GATA-1(low) mice). Blood. 2002;100(4):1123-1132.
5. Bock O, Loch G, Büsche G, von Wasielewski R, Schlué J, Kreipe H. Aberrant expression of platelet-derived growth factor (PDGF) and PDGF receptor-alpha is associated with advanced bone marrow fibrosis in idiopathic myelofibrosis. Haematologica. 2005;90(1):133-134.
6. Bedekovics J, Kiss A, Beke L, Karolyi K, Méhes G. Platelet derived growth factor receptor-beta (PDGFRbeta) expression is limited to activated stromal cells in the bone marrow and shows a strong correlation with the grade of myelofibrosis. Virchows Arch. 2013;463(1):57-65.
7. Gersuk GM, Carmel R, Pattengale PK. Platelet-derived growth factor concentrations in platelet-poor plasma and urine from patients with myeloproliferative disorders. Blood. 1989;74(7):2330-2334.
8. Nurden AT, Nurden P. Inherited thrombocytopenias. Haematologica. 2007;92(9):1158-1164.
9. Turro E, Greene D, Wijgaerts A, et al. A dominant gain-of-function mutation in universal tyrosine kinase SRC causes thrombocytopenia, myelofibrosis, bleeding, and bone pathologies. Sci Transl Med. 2016;8(328):328ra30.
10. Pardanani A, Tefferi A. Imatinib targets other than bcr/abl and their clinical relevance in myeloid disorders. Blood. 2004;104(7):1931-1939.
11. Cools J, DeAngelo DJ, Gotlib J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med. 2003;348(13):1201-1214.
12. Apperley JF, Gardembas M, Melo JV, et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N Engl J Med. 2002;347(7):481-487.
13. Villeval JL, Cohen-Solal K, Tulliez M, et al. High thrombopoietin production by hematopoietic cells induces a fatal myeloproliferative syndrome in mice. Blood. 1997;90(11):4369-4383.
14. Decker M, Martinez-Morentin L, Wang G, et al. Leptin-receptor-expressing bone marrow stromal cells are myofibroblasts in primary myelofibrosis. Nat Cell Biol. 2017;19(6):677-688.
15. Papadopoulos N, Lennartsson J. The PDGF/PDGFR pathway as a drug target. Mol Aspects Med. 2018;62:75-88.
16. Marneth AE, Mullally A. The Molecular Genetics of Myeloproliferative Neoplasms. Cold Spring Harb Perspect Med. 2020;10(2):a034876.
17. Mesa RA. Imatinib and tyrosine kinase inhibition, in the management of BCR-ABL negative myeloproliferative disorders. Biologics. 2007;1(2):129-138.
18. Iurlo A, Gianelli U, Rapezzi D, et al. Imatinib and ruxolitinib association: first experience in two patients. Haematologica. 2014;99(6):e76-77.
19. Stivala S, Codilupi T, Brkic S, et al. Targeting compensatory MEK/ERK activation increases JAK inhibitor efficacy in myeloproliferative neoplasms. J Clin Invest. 2019;129(4):1596-1611.
20. Psaila B, Wang G, Rodriguez-Meira A, et al. Single-Cell Analyses Reveal Megakaryocyte-Biased Hematopoiesis in Myelofibrosis and Identify Mutant Clone-Specific Targets. Mol Cell. 2020;78(3):477-492.e8.