Primary myelofibrosis and its paraneoplastic stromal effects

Ayalew Tefferi
Division of Hematology, Mayo Clinic, Rochester, MN, USA. E-mail: tefferi.ayalew@mayo.edu

During its most recent meeting (November 2006), the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) recommended the use of the name *primary myelofibrosis (PMF)* for the clinicopathologic entity otherwise known as chronic idiopathic myelofibrosis, agnogenic myeloid metaplasia, or myelofibrosis with myeloid metaplasia.[1] The deliberations of the expert panel took into account the fact that the key pathogenetic process in PMF is no longer *idiopathic* or *agnogenic*; PMF is now known to constitute a clonal stem cell disease with recurrent molecular markers (e.g. *JAK2V617F*, *MPLW515L/K*) and/or cytogenetic markers (e.g. del(13q), del (20q), +8, +9, and abnormalities involving chromosome 1, 7, and 12).[2]

The PMF designation also underscores the characteristic, albeit neither invariable nor specific, association of the underlying clonal myeloproliferation with overt myelofibrosis.[3] However, the bone marrow and splenic stromal changes in PMF, including angiogenesis, are reactive in nature and completely reversible with effective treatment of the primary clonal process.[4,5] Such has been the case also with other myeloid neoplasms that are sometimes associated with similar histological changes.[6-9] This, however, does not undermine the possibility of a direct detrimental effect from these paraneoplastic features on both effective hematopoiesis and the tempo of clonal myeloproliferation.

For operational purposes, one can consider two interdependent pathogenetic mechanisms in PMF; a primary megakaryocyte-weighted clonal myeloproliferation and a secondary (paraneoplastic) stromal reaction that includes bone marrow fibrosis, osteosclerosis, angiogenesis, and extramedullary hematopoiesis (EMH). As stated above, current evidence strongly supports the stem cell origin of the clonal myeloproliferation in PMF and this decades-long contention has recently been validated by the demonstration of both cytogenetic and molecular markers of clonality in lymphocytes and myeloid progenitors of patients with PMF.[10-15] What remains at large is the primary clonogenic mutation although both *JAK2V617F* and *MPLW515L/K* are now considered serious candidates in this regard.[16-20] For the record, *JAK2V617F* induces a polycythemia vera-like disease in mice whereas *MPLW515L* causes a PMF phenotype.[18,21-23] Regardless, about half of the patients with PMF do not display either mutation and the precise pathogenetic role of these mutations, when they are present, remains to be clarified. Other molecular alterations in PMF include decreased expression of the tumor suppressor retinoic acid receptor (RAR)-β2 gene as a result of abnormal promoter methylation,[24] and reduced megakaryocyte/platelet surface expression of MPL.[25] Additional insight into the molecular pathogenesis of PMF is currently being pursued through global gene expression analysis.[26,27]

The second component of the pathogenetic process in PMF constitutes the bone marrow stromal changes (i.e. collagen fibrosis, angiogenesis, osteosclerosis) that often accompany the disease as well as EMH that occurs in the spleen, liver, and other non-hepatosplenic sites.[28] These paraneoplastic histological changes are believed to be mediated by clonal cell-derived cytokines as well as autoimmune reactions to the altered bone marrow stroma. Consistent with this notion, bone marrow fibroblasts in PMF have repeatedly been shown to be polyclonal whereas both cellular and extracellular levels of various cytokines are often altered in patients with the disease.[2] It is generally hypothesized that, in humans, an abnormal cellular interaction between megakaryocytes and neutrophils contributes to their release of nosogenic cytokines: transforming growth factor-β (TGF-β), platelet-derived growth factor, basic fibroblast growth factor, and tumor necrosis factor-α.

A similar scenario is considered to occur in experimentally induced myelofibrosis in mice in which either systemic over-expression of thrombopoietin[29-32] or megakaryocyte lineage restricted under-expression of the transcription factor GATA-1[33] results in PMF-like stromal changes. Similar experiments in mice have also suggested a primary fibrogenic role for hematopoietic cell-derived TGF-β[34] and an osteogenic role for stromal cell-derived osteoprotegerin.[35] Finally, there is a current consensus that both circulating progenitor cell trapping and abnormal cytokine stimulation of embryonic hematopoietic sites are implicated as mechanisms of hepatosplenic EMH in PMF.[36-41] Such a contention is supported by the high concordance between cytogenetic findings in bone marrow and splenic tissue in PMF.[42]

In the current issue of the journal, Zetterberg *et al*.[43] describe microvascular density, morphology, and pericyte coverage in the bone marrow of patients with PMF and of mice with experimental myelofibrosis. In both instances, compared to controls, angiogenesis was increased and morphologically aberrant, and the vessels often covered with pericytes. These observations are largely confirmatory; both increased angiogenesis and abnormal vessel structure have previously been reported in the context of both bone marrow and spleen in

patients with PMF.[45-49] Similarly, vascular endothelial growth factor expression by bone marrow cells in such patients has previously been looked into, with similar or different findings.[47,50,51] What was novel in the study by Zetterberg *et al.* was the demonstration of pronounced pericyte coverage of the bone marrow microvasculature in PMF; increased pericyte coverage was also noted in normal bone marrow on vessels with a larger perimeter. Furthermore, the authors demonstrated similar changes in the bone marrow and spleen of mice with experimental myelofibrosis. On the other hand, the informative sample sizes in the study were not large enough to support the authors' suggestion regarding the effect of either *JAK2*V617F or secondary myelofibrosis on bone marrow angiogenesis.

It is unlikely that the above-mentioned observations are unique to PMF and instead probably represent common changes associated with tumor-associated neoangiogenesis, albeit with some tissue-specific differences.[52] Furthermore, the precise pathogenetic contribution of angiogenesis in PMF has not been clarified[53,54] and its prognostic value remains dubious.[44,47] Nevertheless, these limitations do not undermine the potential value of angiogenesis-directed therapy in PMF although proof-of-principle is lacking in this regard.[55,56] In other words, cytokine and immune modulation rather than direct anti-angiogenic activity might underlie the therapeutic efficacy of thalidomide and/or lenalidomide in PMF.[5,57] Whether or not targeting pericytes offers a better anti-angiogenic treatment approach in PMF is unlikely to be addressed any time soon, given the level of current interest in using small molecule drugs against JAK2 and related JAK-STAT constituents.[16]

References

1. Mesa R, Verstovsek S, Cervantes F, Barosi G, Reilly JT, Dupriez B, et al. Primary myelofibrosis (PMF), post polycythemia vera myelofibrosis (post-PV MF), post essential thrombocythemia myelofibrosis (post-ET MF), blast phase PMF (PMF-BP): consensus on terminology by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT). Leuk Res 2007;in press.
2. Tefferi A. Pathogenesis of myelofibrosis with myeloid metaplasia. J Clin Oncol 2005;23:8520-30.
3. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica 2005; 90:1128-32.
4. Deeg HJ, Gooley TA, Flowers ME, Sale GE, Slattery JT, Anasetti C, et al. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. Blood 2003;102:3912-8.
5. Tefferi A, Cortes J, Verstovsek S, Mesa RA, Thomas D, Lasho TL, et al. Lenalidomide therapy in myelofibrosis with myeloid metaplasia. Blood 2006;108:1158-64.
6. Klion AD, Robyn J, Akin C, Noel P, Brown M, Law M, et al. Molecular remission and reversal of myelofibrosis in response to imatinib mesylate treatment in patients with the myeloproliferative variant of hypereosinophilic syndrome. Blood 2004;103:473-8.
7. Beham-Schmid C, Apfelbeck U, Sill H, Tsybrovsky O, Hofler G, Haas OA, et al. Treatment of chronic myelogenous leukemia with the tyrosine kinase inhibitor STI571 results in marked regression of bone marrow fibrosis. Blood 2002;99:381-3.
8. Pardanani A, Brockman SR, Paternoster SF, Flynn HC, Ketterling RP, Lasho TL, et al. FIP1L1-PDGFRA fusion: prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe eosinophilia. Blood 2004;104:3038-45.
9. Bueso-Ramos CE, Cortes J, Talpaz M, O'Brien S, Giles F, Rios MB, et al. Imatinib mesylate therapy reduces bone marrow fibrosis in patients with chronic myelogenous leukemia. Cancer 2004;101:332-6.
10. Reeder TL, Bailey RJ, Dewald GW, Tefferi A. Both B and T lymphocytes may be clonally involved in myelofibrosis with myeloid metaplasia. Blood 2003;101:1981-3.
11. Larsen TS, Christensen JH, Hasselbalch HC, Pallisgaard N. The JAK2 V617F mutation involves B- and T-lymphocyte lineages in a subgroup of patients with Philadelphia-chromosome negative chronic myeloproliferative disorders. Br J Haematol 2007;136:745-51.
12. Bogani C, Guglielmelli P, Antonioli E, Pancrazzi A, Bosi A, Vannucchi AM. B-, T-, and NK-cell lineage involvement in JAK2V617F-positive patients with idiopathic myelofibrosis. Haematologica 2007;92:258-9.
13. Delhommeau F, Dupont S, Tonetti C, Masse A, Godin I, Le Couedic JP, et al. Evidence that the JAK2 G1849T (V617F) mutation occurs in a lymphomyeloid progenitor in polycythemia vera and idiopathic myelofibrosis. Blood 2007; 109:71-7.
14. Florensa L, Bellosillo B, Besses C, Puigdecanet E, Espinet B, Perez-Vila E, et al. JAK2 V617F mutation analysis in different myeloid lineages (granulocytes, platelets, CFU-MK, BFU-E and CFU-GM) in essential thrombocythemia patients. Leukemia 2006;20:1903-5.
15. Ishii T, Bruno E, Hoffman R, Xu M. Involvement of various hematopoietic-cell lineages by the JAK2V617F mutation in polycythemia vera. Blood 2006;108:3128-34.
16. Tefferi A, Gilliland DG. Oncogenes in myeloproliferative disorders. Cell Cycle 2007;6:550-66.
17. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 2005;434:1144-8.
18. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, et al. MPLW515L Is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med 2006;3:e270.
19. Lasho TL, Pardanani A, McClure RF, Mesa RA, Levine RL, Gary Gilliland D, et al. Concurrent MPL515 and JAK2V617F mutations in myelofibrosis: chronology of clonal emergence and changes in mutant allele burden over time. Br J Haematol 2006;135:683-7.
20. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood 2006;108:3472-6.
21. Pikman Y, Lee BH, Mercher T, Wadleigh M, Gilliland DG, Levine RL. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS 2006;e270.
22. Lacout C, Pisani DF, Tulliez M, Moreau Gachelin F, Vainchenker W, et al. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. Blood 2006;108:1652-60.
23. Wernig G, Mercher T, Okabe R, Levine RL, Lee BH, Gilliland DG. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. Blood 2006; 107:4274-81.
24. Jones LC, Tefferi A, Idos GE, Kumagai T, Hofmann WK, Koeffler HP. RARβ2 is a candidate tumor suppressor gene in myelofibrosis with myeloid metaplasia. Oncogene 2004;23:7846-53.
25. Moliterno AR, Hankins WD, Spivak JL. Impaired expression of the thrombopoietin receptor by platelets from patients with polycythemia vera. N Engl J Med 1998; 338: 572-80.
26. Guglielmelli P, Zini R, Bogani C, Salati S, Pancrazzi A, Bianchi E, et al. Molecular profiling of CD34+ cells in idiopathic myelofibrosis identifies a set of disease-associated genes and reveals the clinical significance of Wilms' tumor gene 1 (WT1). Stem Cells 2007;25:165-73.
27. Jones LC, Tefferi A, Vuong PT, Desmond JC, Hofmann

WK, Koeffler HP. Detection of aberrant gene expression in CD34+ hematopoietic stem cells from patients with agnogenic myeloid metaplasia using oligonucleotide microarrays. Stem Cells 2005;23:631-7.
28. Koch CA, Li CY, Mesa RA, Tefferi A. Nonhepatosplenic extramedullary hematopoiesis: associated diseases, pathology, clinical course, and treatment. Mayo Clinic Proc 2003; 78:1223-33.
29. Yan XQ, Lacey D, Fletcher F, Hartley C, McElroy P, Sun Y, et al. Chronic exposure to retroviral vector encoded MGDF (mpl-ligand) induces lineage-specific growth and differentiation of megakaryocytes in mice. Blood 1995;86:4025-33.
30. Yan XQ, Lacey D, Hill D, Chen Y, Fletcher F, Hawley RG, et al. A model of myelofibrosis and osteosclerosis in mice induced by overexpressing thrombopoietin (mpl ligand): reversal of disease by bone marrow transplantation. Blood 1996;88:402-9.
31. Yanagida M, Ide Y, Imai A, Toriyama M, Aoki T, Harada K, et al. The role of transforming growth factor-beta in PEG-rHuMGDF-induced reversible myelofibrosis in rats. Br J Haematol 1997;99:739-45.
32. Villeval JL, Cohensolal K, Tulliez M, Giraudier S, Guichard J, Burstein SA, et al. High thrombopoietin production by hematopoietic cells induces a fatal myeloproliferative syndrome in mice. Blood 1997;90:4369-83.
33. Vannucchi AM, Bianchi L, Cellai C, Paoletti F, Rana RA, Lorenzini R, et al. Development of myelofibrosis in mice genetically impaired for GATA-1 expression (GATA-1$^{(low)}$ mice). Blood 2002;100:1123-32.
34. Chagraoui H, Komura E, Tulliez M, Giraudier S, Vainchenker W, Wendling F. Prominent role of TGF-β 1 in thrombopoietin-induced myelofibrosis in mice. Blood 2002; 100:3495-503.
35. Chagraoui H, Tulliez M, Smayra T, Komura E, Giraudier S, Yun T, et al. Stimulation of osteoprotegerin production is responsible for osteosclerosis in mice overexpressing TPO. Blood 2003;101:2983-9.
36. Wolf BC, Neiman RS. Hypothesis: splenic filtration and the pathogenesis of extramedullary hematopoiesis in agnogenic myeloid metaplasia. Hematol Pathol 1987;1:77-80.
37. Wolfe L, Olivieri N, Sallan D, Colan S, Rose V, Propper R, et al. Prevention of cardiac disease by subcutaneous deferoxamine in patients with thalassemia major. N Engl J Med 1985;312:1600-3.
38. O'Keane JC, Wolf BC, Neiman RS. The pathogenesis of splenic extramedullary hematopoiesis in metastatic carcinoma. Cancer 1989;63:1539-43.
39. Dameshek W. Some speculations on the myeloproliferative syndromes. Blood 1951;6:372-5.
40. Ward HP, Block MH. The natural history of agnogenic myeloid metaplasia (AMM) and a critical evaluation of its relationship with the myeloproliferative syndrome. Medicine 1971;50:357-420.
41. Laszlo J. Myeloproliferative disorders (MPD): myelofibrosis, myelosclerosis, extramedullary hematopoiesis, undifferentiated MPD, and hemorrhagic thrombocythemia. Semin Hematol 1975;12:409-32.
42. Mesa RA, Li CY, Schroeder G, Tefferi A. Clinical correlates of splenic histopathology and splenic karyotype in myelofibrosis with myeloid metaplasia. Blood 2001;97:3665-7.
43. Zetterberg E, Vannucchi AM, Migliaccio AR, Vainchenker W, Tulliez M, Dickie R, et al. Pericyte coverage of abnormal blood vessels in myelofibrotic bone marrows. Haematologica 2007;92:597-604.
44. Mesa RA, Hanson CA, Rajkumar SV, Schroeder G, Tefferi A. Evaluation and clinical correlations of bone marrow angiogenesis in myelofibrosis with myeloid metaplasia. Blood 2000;96:3374-80.
45. Ni H, Barosi G, Hoffman R. Quantitative evaluation of bone marrow angiogenesis in idiopathic myelofibrosis. Am J Clin Pathol 2006;126:241-7.
46. Barosi G, Vittorio R, Margherita M, Luca VG, Alessandro P, Vittorio N, et al. Spleen neoangiogenesis in patients with myelofibrosis with myeloid metaplasia. Br J Haematol 2004;124:618-25.
47. Arora B, Ho CL, Hoyer JD, Mesa RA, Tefferi A. Bone marrow angiogenesis and its clinical correlates in myelofibrosis with myeloid metaplasia. Haematologica 2004;89:1454-8.
48. Kvasnicka HM, Thiele J. Bone marrow angiogenesis: methods of quantification and changes evolving in chronic myeloproliferative disorders. Histol Histopathol 2004;19: 1245-60.
49. Lundberg LG, Lerner R, Sundelin P, Rogers R, Folkman J, Palmblad J. Bone marrow in polycythemia vera, chronic myelocytic leukemia, and myelofibrosis has an increased vascularity. Am J Pathol 2000;157:15-9.
50. Ho CL, Arora B, Hoyer JD, Wellik LE, Mesa RA, Tefferi A. Bone marrow expression of vascular endothelial growth factor in myelofibrosis with myeloid metaplasia. Eur J Haematol 2005;74:35-9.
51. Wrobel T, Mazur G, Surowiak P, Wolowiec D, Jelen M, Kuliczkowsky K. Increased expression of vascular endothelial growth factor (VEGF) in bone marrow of patients with myeloproliferative disorders (MPD). Pathol Oncol Res 2003;9:170-3.
52. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. Circ Res 2005;97:512-23.
53. Chou JM, Li CY, Tefferi A. Bone marrow immunohistochemical studies of angiogenic cytokines and their receptors in myelofibrosis with myeloid metaplasia. Leuk Res 2003;27:499-504.
54. Yoon SY, Tefferi A, Li CY. Bone marrow stromal cell distribution of basic fibroblast growth factor in chronic myeloid disorders. Haematologica 2001;86:52-7.
55. Giles FJ, Cooper MA, Silverman L, Karp JE, Lancet JE, Zangari M, et al. Phase II study of SU5416 - a small-molecule, vascular endothelial growth factor tyrosine-kinase receptor inhibitor - in patients with refractory myeloproliferative diseases. Cancer 2003;97:1920-8.
56. Arora B, Mesa R, Tefferi A. Angiogenesis and anti-angiogenic therapy in myelofibrosis with myeloid metaplasia. Leuk Lymphoma. 2004;45:2373-86.
57. Thomas DA, Giles FJ, Albitar M, Cortes JE, Verstovsek S, Faderl S, et al. Thalidomide therapy for myelofibrosis with myeloid metaplasia. Cancer 2006;106:1974-84.