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Pathogenesis of Myelofibrosis With Myeloid Metaplasia

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ABSTRACT

The primary disease process in myelofibrosis with myeloid metaplasia (MMM) is clonal myeloproliferation with varying degrees of phenotypic differentiation. This is characteristically accompanied by secondary intramedullary collagen fibrosis, osteosclerosis, angiogenesis, and extramedullary hematopoiesis. Modern clonality studies have confirmed the multipotent stem-cell origin of the neoplastic process in MMM. The nature of the specific oncogenic mutation(s) is currently being unraveled with the recent discovery of an association between a somatic point mutation of JAK2 tyrosine kinase (V617F) and bcr/abl-negative myeloproliferative disorders, including MMM. The pathogenetic mechanisms that underlie the secondary bone marrow stromal changes in MMM are also incompletely understood. Mouse models of this latter disease aspect have been constructed by either in vivo overexpression of thrombopoietin (TPO^{high} mice) or megakaryocyte lineage restricted underexpression of the transcription factor GATA-1 ($GATA\text{-}1^{low}$ mice). Gene knockout experiments using such animal models have suggested the essential role of hematopoietic cell-derived transforming growth factor beta1 in inducing bone marrow fibrosis and stromal cell–derived osteoprotegerin in promoting osteosclerosis. However, experimental myelofibrosis in mice does not recapitulate clonal myeloproliferation that is fundamental to human MMM. Other cytokines that are implicated in mediating myelofibrosis and angiogenesis in MMM include basic fibroblast, platelet-derived, and vascular endothelial growth factors. It is currently assumed that such cytokines are abnormally released from clonal megakaryocytes as a result of a pathologic interaction with neutrophils (eg, emperipolesis). This latter phenomenon, through neutrophil-derived elastase, could also underlie the abnormal peripheral-blood egress of myeloid progenitors in MMM.

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INTRODUCTION

Myelofibrosis with myeloid metaplasia (MMM) was first described in 1879[1] and is currently classified as a myeloproliferative disorder (MPD).[2] The disease presents either de novo (agnogenic myeloid metaplasia) or in the setting of both polycythemia vera (PV; postpolycythemic myeloid metaplasia) and essential thrombocythemia (ET; post-thrombocythemic myeloid metaplasia).[3,4] The clinical phenotype includes progressive anemia, massive splenomegaly, a leukoerythroblastic blood smear, profound constitutional symptoms, cachexia, and hepatosplenic as well as nonhepatosplenic extramedullary hematopoiesis.[5] Bone marrow histology displays substantial collagen fibrosis, osteosclerosis, and angiogenesis.[6] Most patients are diagnosed after age 60 years,[7] and reported median survival ranges from 3.5 to 10 years.[8-11] Causes of death include infection, bleeding, organ failure, portal hypertension, and leukemic transformation.[9-11] The latter occurs in 8% to 23% of the patients in the first decade of the disease. Current drug therapy has not altered the natural history of the disease.[12] Although the prospect of cure is possible with allogeneic hematopoietic stem-cell transplantation, the majority of affected patients are not suitable candidates for the particular treatment modality because of either advanced age or the presence of comorbid conditions.[13] Therefore, rational treatment approaches in MMM await elucidation of the underlying pathogenetic mechanisms of both clonal myeloproliferation[14] and the reactive bone marrow stromal changes that are believed to be cytokine mediated.[15]

CLONALITY STUDIES

Normal hematopoiesis in large animals is polyclonal, and individual multipotent stem

cells are capable of both myeloid and lymphoid lineage differentiation.[16] In contrast, hematopoiesis in patients with MPD, including those with MMM, is monoclonal and is supported by a genetically transformed mutant clone that retains the capacity to differentiate across multiple cell lineages.[17] In general, clonality studies in MPD have mostly been based on allelic polymorphisms between the paternally and maternally derived X chromosomes in females.[18] In a normal (polyclonal) somatic cell population, the random X chromosome inactivation process has affected both the maternal and paternal X chromosomes.[19] In a clonal cell population, in contrast, because of the single-cell derivation of the neoplastic process, only the maternal or the paternal X chromosome is affected (not both). This difference in X chromosome inactivation pattern between so-called normal and clonal cell populations can be demonstrated in informative (heterozygous at the genetic locus of interest) females, at either the DNA (on the basis of methylation differences between active and inactive genes),[20] or post-DNA level (on the basis of the fact that RNA and enzyme expression is restricted to the active chromosome).[18,21,22] Some of the classic studies in this regard have used glucose-6-phosphate dehydrogenase isoenzyme analysis (ie, protein level investigation),[14,23,24] whereas more recent studies have focused on X-linked DNA[25,26] and transcript[21] analysis.

Monoclonality in MMM was suggested as early as 1968 based on the clonal occurrence of an abnormal chromosome marker.[27] Subsequent glucose-6-phosphate dehydrogenase–based clonality studies in the 1970s documented clonal involvement of bone marrow mononuclear cells as well as peripheral-blood granulocytes, erythrocytes, and platelets, but not bone marrow fibroblasts.[14,28] Similarly, X-linked DNA analysis has revealed monoclonal X chromosome inactivation pattern in peripheral-blood leukocytes,[29] granulocytes,[26,30] bone marrow mononuclear cells,[26] and, in some cases, T lymphocytes.[30] These observations were supported by cytogenetics-based demonstration of clonality in erythroid,[31,32] granulocyte-monocyte,[31,32] and granulocyte-monocyte-erythroid progenitors,[32] but not in fibroblasts.[33,34] Clonality in MMM has also been investigated using *N*-ras mutation as a marker and showing its presence in granulocytes, monocytes, and erythroblasts, as well as B and T lymphocytes.[35] Similarly, a recent study used a combination of immunomagnetic cell separation technique and interphase cytogenetics to demonstrate clonal involvement of T and B lymphocytes (Fig 1).[17] Therefore, current evidence strongly supports the true stem-cell nature of the mutant clone in MMM, and within the confines of the available testing methods, interpatient heterogeneity in the extent of clonal involvement by a specific cell type has been demonstrated.[17,30]

THE SEARCH FOR DISEASE-SPECIFIC MUTATIONS

Cytogenetic Studies

Despite the abundant evidence for clonal hematopoiesis in MMM, the nature of the disease-causing genetic mutation remains elusive. The detection of a consistent cytogenetic abnormality in myeloid malignancies is not only diagnostically useful,[36,37] but may also provide pathogenetic clues.[38,39] Unfortunately, the majority of myeloid disorders do not bear a specific karyotypic marker, and observed abnormalities often represent secondary subclones possibly linked to the genetic

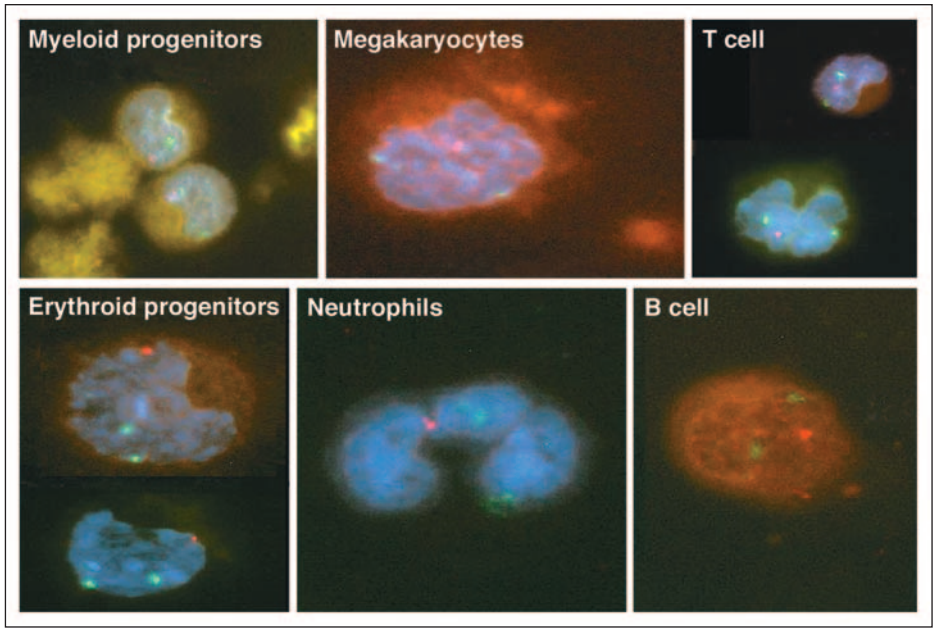

Fig 1. Fluorescent in situ hybridization studies of T ($CD3^+$) and B ($CD19^+$) lymphocytes, neutrophils, and precursor cells of myeloid ($CD34^+$), megakaryocyte ($CD61^+$), and erythroid ($CD71^+$) lineage in myelofibrosis with myeloid metaplasia. All cells exhibit a single orange signal, revealing a deletion of the long arm of chromosome 20/13, and two green signals of the control probe.

instability of the mutant clone.[40,41] Nevertheless, the cytogenetics of MMM has been extensively studied, and a wide spectrum of structural and numerical chromosome abnormalities have been described.[42-63] In general, recurrent cytogenetic abnormalities occur in approximately 50% of chemotherapy-naive patients with MMM, and the most prevalent lesions, representing more than 80% of the abnormal cases, include del(20)(q11;q13), del(13)(q12;q22), trisomy 8, trisomy 9, del(12)(p11;p13), monosomy or long-arm deletions involving chromosome 7, and partial trisomy 1q (Fig 2).[57,63] However, the individual lesions occur in only the minority of patients (10% to 25% of the abnormal cases), and none are specific to MMM (Fig 2).[63] Furthermore, the application of molecular cytogenetic studies by fluorescent in situ hybridization did not disclose additional, karyotypically occult, structural lesions.[64] Incidentally, the pattern of cytogenetic abnormalities is similar between the three subtypes of MMM: agnogenic myeloid metaplasia, postpolycythemic myeloid metaplasia, and postthrombocythemic myeloid metaplasia.[63]

As mentioned above, although not disease specific, 13q-, 20q-, and abnormalities of chromosome 1 are characteristically prevalent in MMM and constitute a focus of interest regarding pathogenetic insight. Molecular characterization of 13q- in *bcr/abl*-negative MPD has revealed microdeletions that did not involve the retinoblastoma (*RB1*) tumor suppressor gene locus,[65] whereas the *RB1* region is commonly deleted in 13q- associated with multiple myeloma.[66,67] Consistent with this observation, the particular cytogenetic abnormality seems to confer good prognosis in MMM but is associated with adverse prognosis in multiple myeloma.[63,68] In regard to 20q-, similar molecular studies have suggested commonly deleted regions that were specific to either MPD or myelodysplastic syndrome/acute myeloid leukemia.[69] Accordingly, further inquiry into the specific genes that are located in the commonly deleted regions of MPD-associated 20q- might shed additional pathogenetic insight on MMM. Partial trisomy 1 that is associated with certain derivative chromosomes is arguably the most specific cytogenetic abnormality in MMM. Interestingly, one particular such lesion, der(6)t(1 ;6)(q21-23;p21-23), involves a chromosomal region (6p21) that houses the FK-506 binding protein 5 (*FKBP51*) gene, which was recently shown to be overexpressed in $CD34^+$ cell-derived megakaryocytes of patients with MMM and potentially confer an antiapoptotic effect through inhibition of calcineurin.[70] Molecular studies are currently underway to map the precise breakpoints of these intriguing translocations and learn their pathogenetic role in MMM.

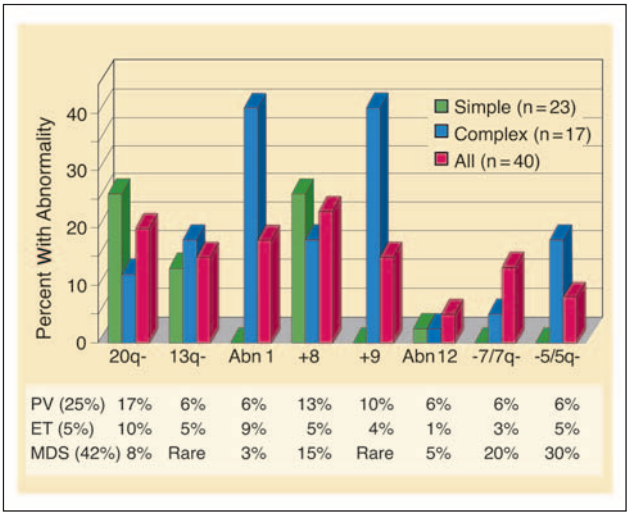

Fig 2. Karyotypic abnormalities (Abns) in 83 chemotherapy-naive patients with myelofibrosis with myeloid metaplasia. For comparison, the prevalence of each anomaly in other chronic myeloid disorders, including polycythemia vera (PV), essential thrombocythemia (ET), and myelodysplastic syndrome (MDS), is provided in the box below the figure.[63,199-201].

Mutation Screening Studies

The pathogenesis of MMM and related *bcr/abl*-negative MPD might be explained, in part, by a somatic point mutation on exon 14 (V617F) of the *JAK2* kinase gene that is located on chromosome 9p24.[71-73] The recurrent *JAK2* mutation was identified by either a candidate gene approach[71,73] or a high-throughput DNA sequencing of the functional domains of 85 human tyrosine kinases.[72] The incidence of the mutant allele in granulocytes collected from MPD patients was 35% to 50% in MMM, 32% to 57% in ET, and 74% to 97% in PV.[72,73] No mutation was detected in either normal individuals or patients with secondary erythrocytosis.[71-73] The *JAK2* V617F mutation occurs within the auto-inhibitory JH2 domain, resulting in dysregulation of kinase activity that resides in the JH1 domain. Supporting evidence for this comes from the demonstration of mutant allele-mediated activation of both JAK2- and STAT5-mediated transcription, as well as the induction of either Epo hypersensitivity or growth factor independence in cell lines.[71,72] Furthermore, erythrocytosis was induced in mice transplanted with bone marrow cells carrying the mutant allele.[71] However, direct mutation screening for other candidate genes, including type III receptor tyrosine kinases (*c-kit, c-fms, flt-3*), has generally yielded negative results.[74,75]

Loss-of-Heterozygosity Studies

In certain human cancers, loss of heterozygosity (LOH) of chromosomal regions harboring mutated tumor suppressor genes is either a key oncogenic event or contributes to disease progression. Genome-wide patterns of LOH can be studied using polymorphic genetic markers, and the principle behind the particular molecular investigation is that the loss of the normal allele of a tumor suppressor gene in the presence of a mutated allele results in homozygous loss of protective gene function.[76]

In a recent study, a genome-wide screening for LOH using 86 polymorphic markers encompassing all 22 autosomes was performed on $CD34^+$ cells obtained from 29 patients with MMM.[77] Allelic loss was compared with that of normal T lymphocytes from the same patients. After combing the results of marker studies on the same arm of individual chromosomes, the most frequent allelic loss was observed on 1q (25%), 3p (24%), and 3q (22%).[77] Interestingly, the LOH patterns were not correlated with structural lesions detected by cytogenetic studies. Using 23 additional LOH markers, a detailed mapping of the chromosome 3p arm between D3S1597 and D3S1578 revealed a CDR between D3S1583 and D3S1609 at 3p24 that houses the retinoic acid receptor tumor suppressor gene. Subsequent experiments revealed that *RAR2* gene expression was markedly decreased in all tested patients with MMM, even in the absence of LOH at the particular locus. Additional experiments suggested that this was the result of an epigenetic mechanism mediated by abnormal methylation of the gene promoter P2.[77] Although RAR is considered important in myeloid differentiation, additional studies are needed to decipher the specific pathogenetic role of the observed phenomenon in MMM. In the meantime, the particular work provides support for evaluating the therapeutic value of demethylating as well as retinoic acid-based agents in MMM.[78,79]

Gene Expression Studies

In gene expression analysis, an altered gene or biochemical pathway associated with a particular disease may be revealed by the identification of a consistently upregulated or downregulated gene across a cohort of patients with the same disease.[80] Global gene expression profiling has been shown to accurately distinguish among previously established phenotypic categories of hematologic malignancies as well as reveal additional molecular classes.[81] More importantly, considerable pathogenetic insight may be obtained by comparing gene expression patterns of diseased and normal tissue.

To date, only one study has evaluated MMM by oligonucleotide microarray analysis.[82] Gene expression by $CD34^+$ cells in eight patients with MMM was compared with that of six normal controls. Upregulated genes in MMM included several transcription factors (*JUNB* at 19p13.2, *GATA2* at 3q21, *N*-myc at 2p24.1), granulocyte-macrophage colony-stimulating factor (*CSF1* at 5q33), interleukin-8 (*IL8* at 4q13-21), *IL1B* at 2q14, platelet-derived endothelial cell growth factor (*PDECGF* at 22q13.33), platelet factor 4 at 4q12-21, and delta, *Drosophila* homolog-like 1 (*DLK1* at 14q32). Downregulated genes included connective tissue growth factor (*CTGF*) that is located on chromosome 6q23.1, osteopontin (*OPN*) located at 4q21-25, FK-506 binding protein 5 (*FKBP5*) located at 6p21, bone morphogenic protein 2B (*BMP2B*) located at 14q22-23, macrophage inhibitory cytokine-1 (*MIC-1*) located at 19p13, *BCL-1* located at 11q13, cell division cycle 2 (*cdc2*) located 10q21.1, and *cdc20* located at 9q13-21.

Some of the upregulated transcription factors (eg, *N*-myc) may be relevant to cell cycle progression and, along with some of the overexpressed growth factors (eg, CSF1, PDECGF) and pleiotropic cytokines (eg, IL-8, IL-1B), might contribute to the myeloproliferation and stromal reaction in MMM.[83-85] Dlk1 is a transmembrane protein belonging to the superfamily of epidermal growth factor–like proteins and is essential for normal hematopoiesis, and its abnormal expression has also been demonstrated in myelodysplastic syndrome.[86] Osteopontin is a secreted cell attachment phosphoprotein that might participate in cell-matrix interaction and might facilitate fibroblast, osteoblast, and osteoclast contribution to the pathology of the stromal reaction in MMM.[87] BMP2B might play a similar role in the associated changes in bone remodeling.[88] The results of the aforementioned DNA microarray study regarding *FKBP51* gene expression is at odds with another study that used a differential display approach and demonstrated overexpression in megakaryocytes derived from $CD34^+$ cells of patients with MMM.[70] The discrepancy might have arisen from either the distinct cell populations studied in the two different reports (peripheral-blood $CD34^+$ cells[82] *v* CD34-derived megakaryocytes[70]) or the associated experimental conditions in the latter study (comparison of cytokine-prepared normal megakaryocytes *v* spontaneously grown MMM megakaryocytes).[70] Regardless, the systematic evaluation of data coming out of various types of gene expression studies, including DNA microarray analysis, should help generate specific hypothesis for more focused molecular investigations.

Other Molecular and Biologic Studies

Another biologic feature of megakaryocytes in MMM is their endogenous (cytokine-independent) growth capability as well as their hypersensitivity to various growth factors, including thrombopoietin (TPO).[89-94] The specific in vitro growth characteristic might also affect other cell lineages[95,96] and is not specific to MMM, as it is readily seen in other MPD, including PV[97-99] and ET.[96,100-102] Such growth factor independence is not seen in either normal subjects or reactive myeloproliferation[103] and, in MPD, has not been attributed to mutations in ligand receptor[89,104] or receptor-associated signal transducer molecules.[105] One suggested mechanism of intrinsic growth factor hypersensitivity in MMM involves megakaryocyte overexpression of *FKBP51* that is associated with constitutive JAK2/STAT5 activation.[70,106] This possibility is consistent with the recent discovery of an activation mutation of JAK2 (V617F) in

MPD.[71-73] However, megakaryocyte *FKBP51* gene as well as protein expression in MMM was shown to be similar to that of normal megakaryocytes in another study.[107]

Another study has suggested the constitutive activation of the γc/JAK3/STAT3 pathway in $CD34^+$ cells from patients with MMM that was coupled with the inability to differentiate into natural-killer cells.[108] Obviously, such examples of altered cellular biology are by no means specific to MMM and could represent a nonspecific feature that is common to many neoplastic cell processes.[109,110] The same can be said about the stem-cell leukemia gene, the expression of which in blood mononuclear cells from patients with MMM was shown to be increased.[111] Markedly decreased megakaryocyte/platelet expression of the TPO receptor (Mpl) is another nonspecific[112,113] cellular feature in MMM[114] that is also seen in ET[115] and PV.[114] The particular phenomenon has been blamed for the normal or elevated serum concentrations of TPO in MMM[116] that is unexpected in lieu of the disease-associated increased megakaryocyte mass.

PATHOGENETIC MECHANISM OF BONE MARROW STROMAL REACTION

MMM is typically characterized by bone marrow collagen fibrosis, new bone formation (osteosclerosis), and angiogenesis.[6,117] As elaborated in the previous section on clonality studies, bone marrow fibroblasts in MMM are polyclonal[33,34,118] and exhibit normal function and in vitro growth characteristics.[118] This information, coupled with the observation that both cellular and extracellular levels of various fibrogenic and angiogenic cytokines are altered in patients with MMM, strongly supports the contention that the bone marrow histologic changes in MMM are reactive and cytokine mediated.[118-120] In this regard, several patient-centered studies have suggested a pathogenetic role for transforming growth factor beta (TGF-β),[121-123] platelet-derived growth factor,[123] basic fibroblast growth factor,[124-126] vascular endothelial growth factor (VEGF),[127] and tissue inhibitors of matrix metalloproteinases.[128,129] Similar circumstantial evidence has long suggested that megakaryocytes[122,130] and activated monocytes[120] might be the source of the aforementioned nosogenic cytokines. Furthermore, a pathologic interaction between megakaryocytes and neutrophils (emperipolesis), induced by altered megakaryocyte P-selectin distribution, might underlie the abnormal cytokine release mechanism.[131,132] Neutrophil-derived enzymes, including elastase, might also participate in the pathologic process by facilitating peripheral-blood egress of myeloid progenitors.[133-135]

Mouse models of myelofibrosis have provided additional information regarding the role of certain cytokines in the pathogenesis of the stromal reaction in MMM.[136,137] There are currently two established models of experimental myelofibrosis in mice: TPO^{high} and GATA-1^{low} mice. The former is constructed by the systemic overexpression of thrombopoietin[137-140] and the latter by megakaryocyte lineage restricted underexpression of the transcription factor GATA-1.[141] The common feature to both experimental models is tissue accumulation of megakaryocytes, a result of TPO-driven proliferation in TPO^{high} mice[142] and impaired megakaryocyte maturation in GATA-1^{low} mice.[143] However, considerable heterogeneity exists among different mouse models in terms of both phenotype and disease tempo (Fig 3).[138,140,141,144-146] In immune-deficient mice, myelofibrosis and osteosclerosis developed only in the severe combined immunodeficient (T and B cell deficient) model attributed to both the high degree of TPO expression, compared with the nude (T cell deficient) model, as well as the retention of intact monocyte-macrophage function, compared with the nonobese diabetic severe combined immunodeficient (T and B as well as multiple other defects in innate immunity, including NK and monocyte-macrophage function) model.[146,147] Furthermore, experimental myelofibrosis in TPO^{high} mice has been shown to be reversible either by transplantation[139] or cessation of the systemic administration of TPO.[148]

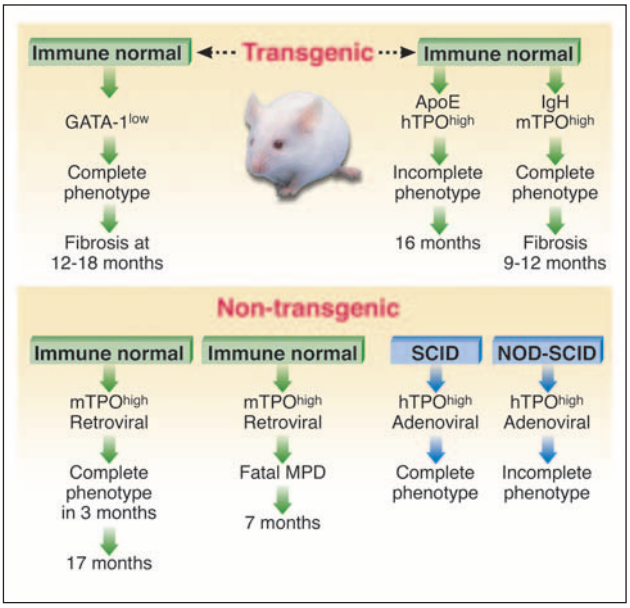

Fig 3. Mouse models of myelofibrosis. Transgenic GATA-1^{low} or IgH/$mTPO^{high}$ develop a complete myelofibrosis with myeloid metaplasia phenotype with delayed onset of myelofibrosis and osteosclerosis.[141,145] In contrast, transgenic ApoE/$hTPO^{high}$ mice develop incomplete phenotype, megakaryocytic hyperplasia without myelofibrosis.[144] Similarly, both complete[138,140,146] and incomplete[146] phenotypes are seen in nontransgenic mouse models whose bone marrow stem cells were retrovirally transduced to overexpress thrombopoietin. SCID, severe combined immunodeficient disease; NOD-SCID, nonobese diabetic severe combined immunodeficient disease.

The role of megakaryocyte-derived TGF-β in myelofibrosis was first suggested by the in vitro observation that the addition of a neutralizing anti–TGF-β antibody inhibited the stimulatory effects of a megakaryoblast-conditioned medium on collagen synthesis in bone marrow fibroblasts from a patient with acute megakaryocytic leukemia.[121] Interestingly, the particular cytokine has also been implicated in hairy cell leukemia–associated reticulin and collagen fibrosis.[149] Consistent with these observations, *TGF-β1* null murine hematopoietic stem cells retrovirally infected with murine *TPO* failed to produce bone marrow fibrosis when transplanted into lethally irradiated wild-type mice, despite the development of all other components of the complete phenotype.[136] Similarly, the osteosclerotic component of experimental myelofibrosis in TPO^{high} mice is aborted by knocking out the stromal cell-derived osteoprotegerin gene.[150] Similarly, an age-dependent increase in osteoprotegerin accompanied the development of osteosclerosis in one of the aforementioned transgenic TPO models.[145] TGF-β1 seems to be similarly important in an the alternative model of GATA-1^{low} mice.[141,151] In this instance, treatment with TPO improved the associated thrombocytopenia as well as stabilized bone marrow fibrosis through downregulation of TGF-β1 expression.[152]

THERAPEUTIC IMPLICATIONS

The aforementioned pathogenetic information regarding the stromal reaction in MMM underlies the rationale for testing a series of drugs at my institution over the last 12 years. Accordingly, specific drugs that have been evaluated included those that were directed at reducing megakaryocyte bulk (interferon alfa-2a,[153] cladribine[154,155]), impairing megakaryocyte differentiation and thus possibly interfering with megakaryocyte-neutrophil interaction (anagrelide[156]), inhibiting TGF-β–mediated fibroblast proliferation and collagen synthesis (suramin,[157] pirfenidone[158]), inhibiting platelet-derived growth factor receptor–associated tyrosine kinase activity (imatinib mesylate[159]), and interfering with angiogenesis and TNF-α production (thalidomide,[160-163] etanercept[164]). Unfortunately, none of these agents were shown to induce favorable changes in bone marrow stroma, although clinical benefit was demonstrated with cladribine, thalidomide, and etanercept.[155,160,164] On the basis of these findings, we are currently running treatment trials with a more potent thalidomide analog (CC-5013; lenalidomide; Revlimid; Celgene, Summit, NJ) as well as combination therapy with thalidomide and etanercept. Revlimid is the lead compound among the immunomodulatory analogs of thalidomide (ImiDs), and its ex vivo antiangiogenic as well as anti-tumor necrosis factor property is estimated to be at least 50-fold higher than that of thalidomide.[165,166] Within the context of myeloid malignancies, the drug was recently shown to have excellent therapeutic activity in MDS associated with a 5q- chromosomal abnormality.[167]

There are several other antiangiogenic agents that might be considered for future therapeutic trials in MMM. In this regard, we have recently completed a phase II study (unpublished) with a farnesyl transferase inhibitor (R115777) that is known to downregulate VEGF expression.[168] Some promise of therapeutic activity in this regard has already been communicated by other investigators.[169] In another upcoming pilot study, we plan to evaluate the therapeutic activity of bortezomib,[170] a proteosome inhibitor, which in addition to its indirect antiangiogenic effect, might overcome the antiapoptotic effect of *FKBP51* overexpression in MMM.[70,106] Other antiangiogenic agents that are undergoing clinical trials and are potent inhibitors of VEGF receptors, platelet-derived growth factor receptor, and Kit include SU-5416, SU-6668, and PTK-787.[171-173] In general, these agents were well tolerated, but their activity has so far been underwhelming.[171,173] Rapamycin is another antiangiogenic drug[174] that might be worth looking into because it also interferes with collagen synthesis[175] and abnormal granulocyte migration.[135,176]

Another venue of therapeutic trials might target the downstream effectors of TGF-β.[177] One such candidate molecule is connective tissue growth factor,[178,179] and human monoclonal antibodies against connective tissue growth factor are already being evaluated in clinical trials involving other fibrotic disorders.[180] One can also envision treatment molecules that target either the TGF-β receptor[181,182] or the postreceptor signaling intermediates, the Smad proteins.[183,184] In the end, it is likely that a combination of drugs directed at different molecules might be needed for effective control of the composite stromal aberration in MMM. In this regard, the recently discovered, MPD-associated JAK2 V617F might constitute a molecular target for the development of rational drug therapy.[185]

In summary, based on the composite set of information obtained from laboratory investigation of patient samples as well as experimental myelofibrosis in mice, a working model of the stromal reaction in MMM is proposed (Fig 4). In this model, the central event is the accumulation of bone marrow megakaryocytes that is clonal[17] in man but cytokine-mediated in experimental myelofibrosis.[137-141] These megakaryocytes, both in man and mice, are also qualitatively abnormal and exhibit abnormal distribution of P-selectin[131,132] and decreased expression of Mpl.[114] The former abnormality promotes a pathologic interaction with neutrophils (emperipolesis) and the latter an in situ increase in TPO concentration. The excess TPO might enhance the underlying clonal myeloproliferation as well as induce stromal cells to produce fibrogenic, osteogenic, and angiogenic cytokines. Such cytokines are also

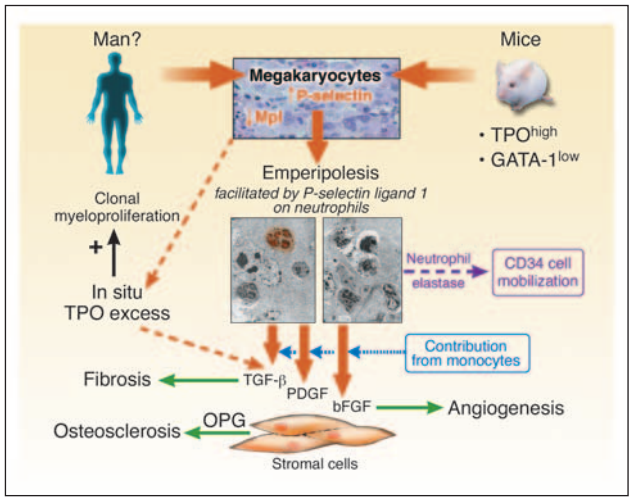

Fig 4. A working model for the pathogenesis of myelofibrosis with myeloid metaplasia. In both the human disease and experimental myelofibrosis in mice, a pathologic interaction between the quantitatively as well as biologically altered megakaryocytes and neutrophils results in abnormal release of fibrogenic and angiogenic cytokines that mediate the bone marrow stromal reaction. TGF-β, transforming growth factor beta; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; OPG, osteoprotegerin.

abnormally released as a result of the aforementioned emperipolesis as well as activation of clonal monocytes.[120] The different components of the overall stromal reaction (fibrosis, osteosclerosis, angiogenesis, CD34 cell egress) might be linked to one or more specific cytokines.[136,150]

It is underscored that experimental myelofibrosis in mice does not recapitulate clonal myeloproliferation that is fundamental to human MMM.[17] However, it does capture the stromal aberration that is integral to the human disease.[186-193] Similarly, alterations in either gene structure or gene expression involving *TPO*,[194] *c-Mpl*,[89,195] *GATA-1*,[196,197] or *FOG-1* (the transcriptional regulator of GATA-1)[198] have not been detected in human MMM. Regardless, the availability of animal models of myelofibrosis should facilitate in vivo preclinical drug testing as well as identification of crucial molecules that can be therapeutically targeted.

Author's Disclosures of Potential Conflicts of Interest

The author indicated no potential conflicts of interest.

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