Pathogenesis of myeloproliferative neoplasms

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A B S T R A C T

Recent advances in our understanding of the pathogenesis of classical myeloproliferative neoplasms (MPNs) – Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Primary Myelofibrosis (PMF) – have led to the identification of oncogenic mutations affecting JAK2 or MPL, leading to constitutive activation of cytokine-regulated intracellular signaling pathways. More recently, numerous other mutations have been found, which affect signaling pathways, such as LNK and CBL, epigenetic regulators, such as TET2, DNMT3A, ASXL1, and EZH2, and spliceosome proteins, such as SF3B1. None of these mutations is specific for MPNs since they are found with high frequency in myelodysplastic syndromes. They are more frequently observed in PMF with a more complex combination of mutations than in PV and ET. A tempting hypothesis is that by affecting stem cell biology, they facilitate the clonal selection and the probability to develop a disease. In addition, they might be implicated in the myelodysplastic features seen in PMF and may induce gene instability favoring Acute Myeloid Leukemia (AML) transformation. However, the MPN phenotype is mainly mediated by an abnormal activation of JAK2 signaling. Of great interest, the *JAK2* locus is implicated in predisposition to MPNs. Identification of new mutations in MPNs, as well as the determination of the clonal hierarchy, will be important to understand the pathogenesis of these heterogeneous disorders better and to develop therapeutic approaches.

Introduction

Myeloproliferative neoplasms (MPNs), previously called myeloproliferative disorders, include several entities initially characterized on their clinical and biological features.[1] The term "myeloproliferative disorders" was originally introduced by William Dameshek in 1951, to describe four different diseases with clinical and biological similarities: Polycythemia Vera (PV), Essential Thrombocythemia (ET), Primary Myelofibrosis (PMF), and Chronic Myeloid Leukemia (CML).[2] Later on, chronic neutrophil leukemia, hypereosinophilic syndromes (HES), systemic mastocytosis, atypical chronic myeloid leukemia, and other rare unclassifiable chronic hemopathies have been included in the MPN classification by the WHO organization.[1] MPNs correspond to clonal disorders emerging from the hematopoietic stem cell compartment. All are characterized by an increased production of mature myeloid blood cells with no marked qualitative defect during terminal differentiation. This last criteria distinguishes MPNs from myelodysplastic syndrome (MDS) and MDS/MPN, in which both a myeloproliferation and a myelodysplasia are present, such as the chronic myelomonocytic leukemia (CMML).

CML represents the paradigm of MPNs because it is a clear clinical and molecular entity. Among Philadelphia-negative MPNs, PV, ET, and PMF are the three most common entities. PV is characterized by an increased erythroid red cell mass with a trilineage proliferation. ET is defined by thrombocytosis (≥450×10^9/L) without increased cell mass and megakaryocyte (MK) hyperplasia in the marrow without myelofibrosis. PMF is histologically defined by the presence of a marked reticulin and collagen fibrosis associated with myeloid and megakaryocyte (MK) proliferation. The clinical and biological characteristics are splenomegaly with the presence of leukoerythroblastosis and the presence of MKs with hypolobulated and cloud-like nuclei. Progression is associated with anemia and cytopenia. ET, PV, and PMF are three clinical entities, but are quite close because ET and PV can also progress to myelofibrosis (secondary myelofibosis) and ET may progress to PV. It has been suggested that some PMF can clinically mimic an ET before the development of a fibrosis, but some abnormal MKs are already present.[3,4] This syndrome has been called pre-fibrotic myelofibrosis. PV, ET, and PMF have some features in common, such as the frequencies of thrombotic or hemorrhage complications and the increased frequency of acute myeloid leukemia, especially for PMF (10-year risk transformation from 12% to 31% according to the risk stratification).[5,6]

The molecular pathogenesis of Ph1-negative MPNs was unknown until 2005, but it was assumed that the pathogenesis of these three disorders would be simple and depend on a single driver genetic event as in CML. In

addition, the demonstration that progenitors of patients with PV, and also with ET and PMF, exhibited cytokine hypersensibility leading to the development of erythroid colonies in the absence of erythropoietin (EPO) strongly suggested that, as in CML, a genetic alteration in signaling pathways controlling blood cell production was at the origin of these disorders.[7] As expected, there is now compelling evidence that classical MPNs depend on a driver event, which leads to a deregulation of JAK2 signaling.[8] However, the genetic complexity of these disorders, including several genetic events deregulating signaling pathways, epigenetic controls of gene expression, and eventually the splicing machinery was totally unexpected (Figure 1). As none of these complex patterns of mutations is specific of MPNs, it is presently unknown whether this genetic complexity is related to the usual heterogeneity of myeloid malignancies that include a large spectrum of overlapping disorders and/or to the weakness of oncogenic drivers, such as JAK2V617F, that require additional genetic events to induce a full blown clonal disorder in humans.

Mutations that activate cytokine signaling

JAK2V617F mutation

JAK2 was an obvious candidate because it plays a central role in myeloid cytokine signaling and in cytokine receptor trafficking, which can be altered in MPNs. In addition, a 9p loss of heterozygocity (LOH), a chromosomal region that includes *JAK2*, was observed in 30% of PV.[9] In 2005, an acquired mutation (a guanine to thymine change at nucleotide 1849 of the cDNA) in exon 14 of the *JAK2* gene leading to a substitution of a valine by a phenylalanine at position 617 was identified in more than 95% of PV, a little more than 50% in ET and PMF, in 30 to 50% of splanchic thrombosis, and in sideroblastic anemia associated with a thrombocytosis. This discovery was important progress in the understanding of the pathogenesis of MPN.[10-13]

This mutation is located in the pseudo-kinase domain (JH2) of JAK2. The JH2 domain negatively regulates the kinase domain (JH1).[14,15] It was assumed that the JH2 domain was catalytically inactive, but recent evidence

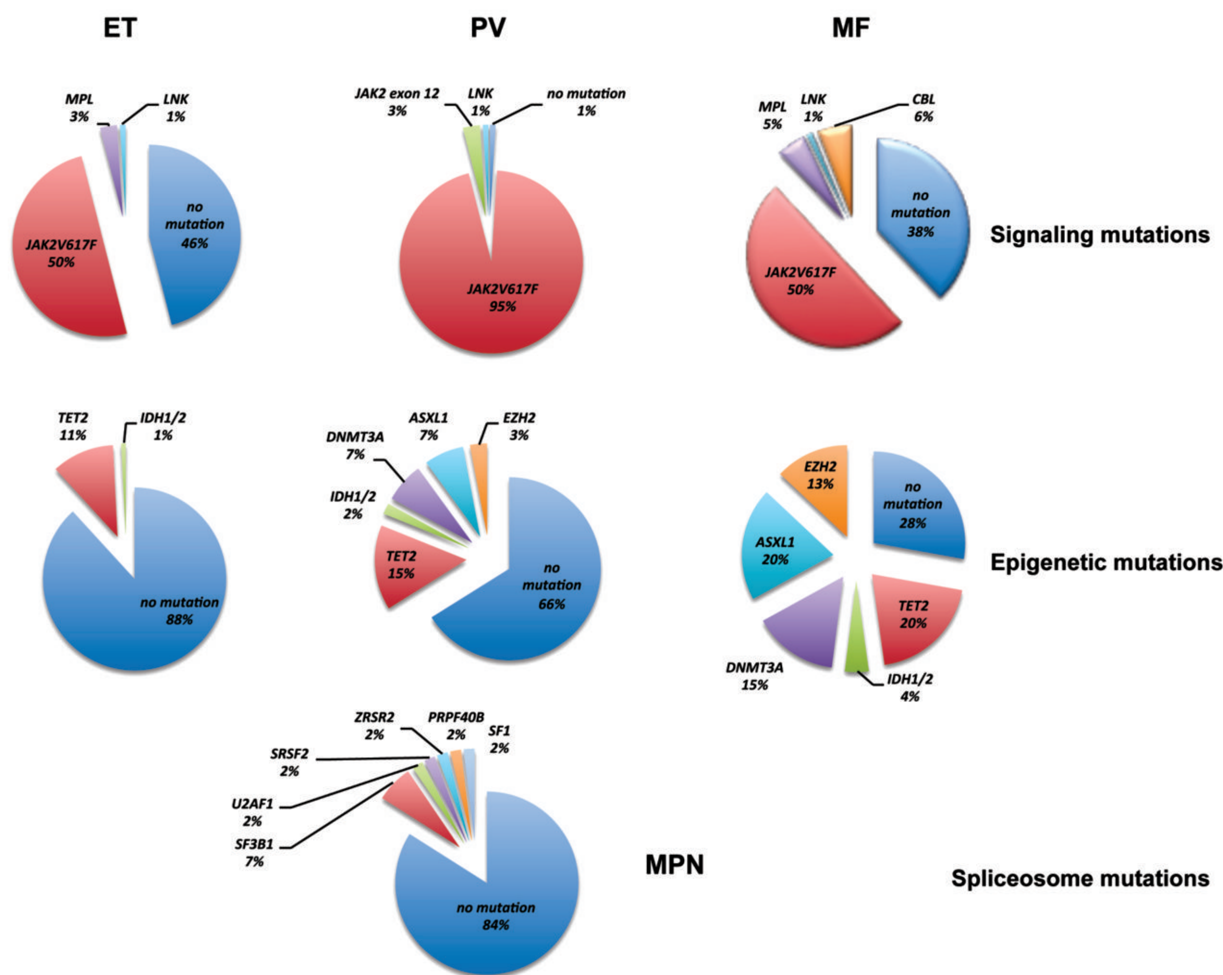

Figure 1. Frequencies and distributions of signaling, epigenetic regulator, and spliceosome pathway mutations among Myeloproliferative Neoplasms (MPNs). For the spliceosome pathway, mutations have been quoted for all MPNs together.

suggests that it behaves as a dual-specificity protein kinase that phosphorylates two negative regulatory sites in JAK2.[15] The V617F mutation, as well as the K539L in exon 12, abrogates this negative effect, leading to increased basal activity.[15,16] Nevertheless, this pre-activated mutant kinase requires a dimeric receptor to induce signaling, especially when expressed at low level. In hematopoiesis, these receptors are G-CSFR, EPOR, and MPL and their expression profile explains why JAK2V617F-induced proliferation affects three myeloid lineages.[17] Artificial JAK2 molecules, such as V617I or V617L that may be obtained by a single nucleotide substitution from the Valine codon, also result in an activated JAK2, but weaker than V617F.[18]

JAK2V617F leads to activation of the canonical signaling pathways of cytokine receptors mainly STATs 5, 3, and 1 depending on the cytokine receptors, the MAP kinase (ERK1 and 2), and the PI3 kinase. Among these pathways, activation of STAT5 plays a central role in cytokine hypersensibility, as well as in the development of the disease.[19,20] The PI3K activation appears to play a synergistic role with STAT5 activation and may be involved in the genetic instability induced by JAK2V617F.[21] JAK2V617F expression also induces hypersensitivity to insulin-like growth factor 1 (IGF1).[22]

More recently, it has been reported that JAK2V617F may activate non-canonical signaling pathways, some directly in the nucleus. Through tyrosine phosphorylation of histone H3, HP1α is excluded from chromatin inducing aberrant gene expression and gene instability.[23] Through phosphorylation of PRMT5, the ability of PRMT5 to methylate histones H2A and H4 is impaired.[24] Thus, JAK2V617F may be involved in epigenetic regulation.

JAK2V617F can be considered as a pre-activated JAK2 molecule, but the mutant has acquired some apparently specific properties: a resistance to SOCS3 negative regulation;[25] an ability to interact with PRMT5;[24] a decreased activity for promoting MPL cell surface expression[26,27](Pecquet *et al.*, manuscript submitted); a preferred association with HSP90 suggesting that targeting HSP90 by destabilizing JAK2V617F is a therapeutic approach;[28] and a specific requirement for the F595 residue for activation of signaling.[29] However, some of these properties, such as resistance to SOCS3, may be related to its deregulated kinase activity.[30] It has to be emphasized that the down-regulation of MPL on the surface of megakaryocytes and platelets might be an important mechanism to escape the anti-proliferative effect of TPO and to induce a thrombocytosis.[31]

Animal model studies have shown that JAK2V617F is indeed able to drive the disease in mice. All mouse models of JAK2V617F expression develop MPN phenotype, usually a PV progressing to myelofibrosis or an ET-like disorder in some other models.[32-39] This latter phenotype corresponds to weak JAK2V617F expression supporting the hypothesis that phenotypic heterogeneity of the JAK2V617F-induced natural diseases might be due to the intensity of JAK2 signaling, especially to the number of JAK2V617F copies.[36] This mimics in part the human situation in which most ET carry a single JAK2V617F mutation whereas two copies of JAK2V617F are present in most PV cases due to uniparental disomy.[40,41]

JAK2 exon 12 mutations

Different somatic gain-of-function mutations in exon 12 of *JAK2* have been found in a fraction of JAK2V617F negative PV.[42,43] These mutations are usually complex, but essentially affect a single residue (K539L mutant). Although not located in the pseudokinase domain, they may modify the structure of the JH2 domain very similarly to V617F.[29] In contrast to JAK2V617F, exon 12 mutations are not associated with ET and PMF. The majority of JAK2 exon 12 PV patients exhibit the clinical features of an idiopathic erythrocytosis,[42,44] but with a trilineage hyperplasia in the marrow.[44] These patients may also progress to a secondary myelofibrosis.[44]

Interestingly, mutations targeting residue R683 of JAK2, which is located in the hinge region between the N- and C-lobes of the pseudokinase domain have been described in Down syndrome and pediatric B cell ALL, but not in MPN.[45-47] In contrast, JAK2V617F or exon 12 mutations are almost specific for MPN. This may be related to different specific interactions between the different mutated JAK2 and cytokine receptors, EpoR, MPL, and G-CSFR for MPN and CRLF2 for ALL.[48,49]

MPL mutations

Several activating mutations of MPL have been found. Most involve the W515 in the exon 10 resulting in its substitution to a leucine, lysine, asparagine, or alanine.[50-54] Amino acid 515 is located in an amphipathic motif (K/RWQFP) found inside the cytoplasm just after the transmembrane domain, which prevents spontaneous activation of the receptor.[54,55] In addition, the MPLS505N mutation, initially described in familial ET,[56] was also found in sporadic MPN.[51,53] Overall MPL mutations have been found in up to 15% of JAK2V617F negative ET or PMF.[57] Bone marrow transplantation assays in mice with W515L or W515A mutations led to an ET-like disorder rapidly progressing to myelofibrosis.[50,54] However, this model is not totally adequate to model the disorder as it results in an ectopic MPL expression, which can induce other phenotypes, such as erythrocytosis.[58,59] *Knock-In* models will be important to understand if MPL mutants can give rise directly to a PMF-like disorder in contrast to JAK2V617F.

LNK

LNK, also called SH2B3, is a member of the SH2B family, which contains two other members.[60] LNK plays an important role in hematopoiesis by negatively regulating JAK2 activation through its SH2 domain.[61,62] As expected from its negative roles on JAK2 signaling, LNK is also capable to attenuate the signaling induced by MPLW515L or JAK2V617F.[63] In JAK2V617F patients, LNK expression is increased and constrains the myeloproliferative disease.[64]

Loss-of-function mutations have been observed in rare MPNs, both in chronic or acute phases.[65,66] These mutations are usually found in the pleckstrin homology domains in a hot spot region located in exon 2, but may also lie outside this exon.[67] Some of these mutations appear to be late events involved in disease progression.[66] In addition, some *LNK* mutations are associated with JAK2V617F, suggesting cooperation between these mutants as in the murine models.[68] *LNK* mutations were initially described in rare ET and PMF, but *LNK* exon 2 mutations have now been reported in pure erythrocytosis.[69]

CBL

The Casitas B-cell lymphoma (CBL) proteins are multifunctional adaptor proteins with ubiquitin ligase activity and major regulatory roles in receptor tyrosine kinase (RTK) negative regulation. However CBL may have numerous targets other than RTK, including JAK2, STAT5, and cytokine receptors, such as MPL.[70] *CBL* is located at 11q.23.3 and is mutated in a variety of myeloid malignancies[71,72] with the highest frequency in CMML and juvenile myelo-monocytic leukemia. *CBL* mutations have also been found in MPN, but only in a low percentage of PMF (6%).[73] A CBL mutation has been detected in blasts from a JAK2V617F positive MPN, which became JAK2V617F negative during transformation.[74]

SOCS 1, 2, and 3

SOCS proteins are also important negative regulators of JAK signaling through a classical feedback loop.[75] Mutations in the different SOCS have been found in MPNs, but infrequently.[76] In contrast, hypermethylation of CpG islands in SOCS1 and SOCS3 associated with a decrease in expression was described in both JAK2V617F-positive and -negative MPNs.[77,78] The role of SOCS proteins in the pathogenesis of MPN remains unclear, especially concerning the role of epigenetic silencing as cooperating event with JAK2V617F[79] or as driving some MPNs, such as ET.

Mutations in epigenetic regulators

There are two main types of epigenetic modifications: the first one concerns DNA methylation at the 5-position of cytosine (mC), and the second one involves histone modifications. The regulation of both processes can be affected by mutations in MPNs. However, none of these mutations are specific of MPNs and are found in a wide range of malignancies, especially in MDS and MDS/MPN. Furthermore, although their functional consequences in MPNs remain only partially understood, these mutations are more frequently found in PMF and during disease progression.

Mutations affecting DNA methylation

TET2

DNA methylation is mediated by DNA methyltransferases (DNMTs). During embryogenesis and differentiation, the genome is constantly methylated and demethylated. Recent studies suggest that DNA methylation may play a role in both gene repression and activation. Until recently, it was assumed that demethylation was mainly passive and related to replication. The discovery of the Ten-Eleven-Translocation (*TET*) gene family through their implication in myeloid malignancies and subsequently the identification of their function have been a great advance in understanding both the mechanisms of active and passive DNA demethylation and its role in development, differentiation, and transformation.

The founding member of the TET family is TET1, which has been isolated as a fusion partner of MLL, in rare t(10;11)(q21;q32) chromosomal translocation of acute leukemia.[80,81] This family includes three proteins (TET1, TET2, and TET3) that are 2-oxoglutarate- and Fe (II)-dependent hydroxylases. They are able to hydroxylate mC into 5-hydroxymethylcytosine (hmC).[82-84] The hmC appears to be an intermediate and can be either further oxidized by the TET proteins into 5-formylcytosine (fC)-and then to 5-carboxylcytosine (caC)[85,86] or deaminated by the AID/APOBEC family into 5-hydroxymethyluracil (hmU).[87,88] Subsequently, the thymine DNA glycosylase (TDG) or the single-strand selective monofunctional uracil-DNA glycosylase 1 (SMUG1) involved in the Base Excision Repair (BER) pathway can glycosylate caC and hmU and replace the modified base by a cytosine.[85] Thus, TET proteins play a central role in the dynamics of DNA epigenetic modifications.[89,90] It remains unclear if hmC have a function by itself and if TET proteins have other functions than mC hydroxylation. Recent evidence demonstrates that TET proteins have a dual function in gene expression (repressive and activating functions) and play a major role in embryogenesis and stem cell biology.[82,91]

TET2 is located in 4q24 and has nine coding exons. *TET2* is mutated in a large spectrum of myeloid malignancies.[92-95] Observed mutations through small insertions or deletions and nonsense mutations mainly result in the loss-of-function of the protein. In the majority of cases, mutations are found on a single allele. Accordingly, patients with *TET2* mutations have lower global hmC.[96,97] *TET2* mutations are found in all types of MPNs (JAK2V617F positive or negative), but with a higher frequency in PMF (around 20%). In the majority of cases, *TET2* mutations occur before *JAK2* V617F in MPNs, but the opposite can be observed.[98,99] In the latter situation, it can be associated with disease progression, including to AML. Mutations on the second *TET2* copy have been also observed during transformation.[99] However, there is no clear evidence that *TET2* mutations in PMF hold a prognostic significance.[100] *TET2* mutations are present in hematopoietic stem cell/progenitor population[93] and endow the mutated HSC with a competitive advantage in xenograft assays.[93] In addition, *TET2* mutations and JAK2V617F act synergistically to induce clonal dominance at the level of hematopoietic progenitors in PV patients.[93,98]

Recently, four teams have reported the effects of Tet2 deficiency in mouse models.[101-104] The phenotype observed was quite similar in all models with both the presence of an increased HSC and progenitor compartments along with differentiation abnormalities in all lineages, including the lymphoid lineages. This phenotype was observed in animals with a single copy deficiency, and the phenotype was slightly increased if the two copies were deficient. The HSC displayed a competitive advantage on normal HSC with increased self-renewal capacities. Animals progressively developed a myeloid disease beginning 4 to 6 months after the deletion with different penetrance depending on the mouse model. In most cases, the animal developed a disease mimicking human CMML. It is noteworthy that up to 60% of *TET2* mutations have been described in CMML. Silencing of *TET2* has been also obtained *in vitro* by shRNAs both in mouse[96,105] and human.[97] *TET2* extinction clearly increases the monocytic differentiation in presence of GM-CSF,[96,97,105] delays hematopoietic differentiation with an excess of immature forms,[105] and induces a defect in erythroid and megakaryocyte differentiation.[97] However, the precise set of genes regulated by TET2 in the HSC biology and differentiation remains to be identified.

IDH1/2

In MPNs, mutations of *IDH1* and *IDH2* have been essentially described during transformation.[106] This is quite intriguing as these mutants may affect the same pathway as TET2 loss. Indeed, it has been shown that mutated IDH1 and IDH2 are neomorphic enzymes that catalyze the reduction of αKG to (R)-2-hydroxyglutarate (2HG). This overproduction of 2HG affects the function of αKG-dependent enzymes, such as TET2, and results in an abnormal hypermethylation phenotype in AML.[105]

DNMT3A

Loss-of-function mutations of *DNMT3A*, previously found in AML and MDS,[107] have been described with a low frequency in MPNs, especially in PMF (15%) and in secondary AML (14%).[108,109] *DNMT3A* mutations are rare in PV (<7%) and in ET. The presence of loss-of-function mutations of *TET2* and *DNMT3A* in the same disorders is unexpected, as they induce opposite effects on DNA methylation. However, in both cases, aberrant methylation occurs in CpG islands of a set of genes regulating HSC properties and differentiation.[110,111]

Mutation affecting histone modifications

ASXL1

ASXL1 is a polycomb gene required for long-term repression of the *HOX* genes[108,112] in Drosophila melanogaster. It is present in a complex that deubiquitinates histone H2A.[113] It is yet unknown if *ASXL1* has a similar function in mammals. *ASXL1* knockout mice have a mild defect in hematopoiesis,[114] but it is unknown if the *ASXL1* mutations lead to a null allele. Mutations in *ASXL1* are frameshift and stop mutations located within the 12th exon of the gene that affect mostly only one copy of the gene and result in the loss of the carboxyterminal PHD domain.[115,116] *ASXL1* mutations are essentially found in late MDS, AML, and in CMML. In MPNs, *ASXL1* mutations are rare in ET and PV (less than 7%), but frequent in PMF (from 19 to 40%).[116,117] It remains controversial if myelofibrosis patients with *ASXL1* mutations have a poor clinical outcome.[118]

Mutations affecting the Polycomb repressive complex 2

The PRC2 is involved in various cellular processes, including proliferation, differentiation, cell identity maintenance, ageing, and plasticity.[119,120] PRC2 includes, in addition to EZH1 or EZH2, EED, RbAp46/48, SUZ12, AEBP2, JARID2, and PCL. PRC2 methylates histone H3 at lysine 27, a mark of transcriptionally inactive chromatin.[121] It also associates to DNMT proteins through the EZH proteins to direct DNA methylation. In addition, the repressive effect of TET proteins on gene expression may be mediated by cooperation with PRC2, probably by facilitating PRC2 recruitment.[122] PRC2 may behave as a tumor suppressor gene for the myeloid series.

EZH2

EZH2 codes for one of the two catalytic subunits of PRC2 and are methyltransferases involved in the di- and tri-methylation of K27.[121,123,124]

EZH2 is mutated in B-cell lymphomas, but the recurrent mutation results in a gain-of-function.[125] In contrast, *EZH2* mutations in myeloid disorders are predicted to be loss-of-function mutations. They may be associated with LOH or deletion of the other copy.[126-128] *EZH2* mutations are rare in MPNs and are observed in PMF or secondary myelofibrosis (about 6%).[118,129] They can be associated with mutations in epigenetic regulators, such as ASXL1 and TET2, CBL, or JAK2V617F. There is evidence that mutant EZH2 is associated with a poor prognosis.[129]

Others

Rare mutations or deletion in other members of the PRC2 complex, such as SUZ12, EED, and JARID2, have been described in MPN.[130] They seem to be more frequent during AML transformation.[131]

In addition, *L3MBTL1*, a polycomb gene, may be one important lost genes in chromosome 20q deletion frequently seen in MPN.[132]

Recently it has been shown that in a mouse model the loss of Bmi1, a component of PRC1, together with ink4a-Arf leads to a myelofibrosis with an abnormal megakaryopoiesis. This is associated with an overexpression of HMGA2, a direct target of Bmi1.[133] Such an overexpression has been previously observed in human PMF, as well as some truncated forms.[134,135] EZH2 deletion may also lead to an overexpression of HMGA2.[133] However, in mouse models, overexpression of HMGA2 alone does not induce a myelofibrosis.[133,136]

Mutations in the splicing machinery

A high frequency of mutations in genes involved in RNA splicing machinery has recently been demonstrated in MDS (45 to 85%).[137] These mutations are rare in MPNs (about 10%).[137] *SF3B1* mutations were observed in 6.5% of myelofibrosis and 3% of ET, and were associated with ring sideroblasts as in MDS.[138,139] *SF3B1* mutations are also found in refractory anemia with sideroblasts and thrombocytosis, a disease also associated with JAK2V617F.[140] Altogether these mutations may inhibit proliferation and induce apoptosis,[137] but mutations in SF3B1 seem less deleterious than in U2AF35.[137,139]

Mutations associated with secondary AML

Other mutations have essentially been observed during transformation of MPNs into AML. Among them, deletions in the *IKZF1* gene are relatively frequent (6/29 post-MPN AML)[141] and are late events. Certain N-RAS mutations have also been found in post-MPN AML, but in some cases the mutation could be found in the chronic phase. *NF1* deletions were mainly found in myelofibrosis and rarely in PV or ET.[142] Their frequency in leukemic transformation is unknown.

Two important genes are *TP53* and *RUNX1(runt related transcription factor 1). TP53* mutations are not associated with the chronic phase of MPNs. However, mutated TP53 have been found with a 20% frequency in post-MPN AML patients.[74,143] In one particular case with both *MPL* and *TET2* mutations during chronic phase ET, multiple *TP53* mutations were identified at the time of post-ET AML that resulted in an oligoclonal pattern in progenitors with one selected subclone, predominantly contributing to the leukemic blasts.[143] Thus, the picture of *TP53* mutations in post-MPN AMLs suggests that they play a promi-

nent role in the transformation process. Furthermore, a functional inactivation of p53 through the mTOR pathway that may play an important role in cytokine hypersensibility was reported during chronic phase MPN.[21] The *RUNX1* gene that encodes a transcription factor with major role in hematopoiesis was found mutated in MDS/AML.[144] In a series of 16 post-MPN AMLs, *RUNX1* mutations mostly targeting the RUNT domain (residues 50 to 177) were found in leukemic blasts of six patients.[74] In the chronic phase, overexpression of RUNX1 has been described in JAK2V617F MPNs, leading to an increase expression of NF-E2.[145]

Modifications of micro RNA (miRNA) expression

In addition to gene mutations, numerous abnormalities of miRNAs have been described in MPNs.[146,147] Presently this deregulated expression seems to be more the consequence of defects in signaling pathways and transcription factors than to gene mutations or deletions as described in the 5q syndrome.[148] Two of them have been directly implicated in the pathogenesis of the MPNs. Overexpression of miR28 and miR16-2 is involved in the downregulation of MPL[149] and erythroid expansion,[150] respectively.

Familial predisposition

On the one hand, it has been demonstrated that there is a modest increased risk (about 5-7 fold) of MPNs among first-degree relatives of MPN patients;[151] on the other hand, there is true familial forms of MPNs with mendelian transmission.[152] These familial forms are very heterogeneous since the same type of MPNs (ET or PV) is observed in some cases whereas different types of MPNs are observed in other cases that may be also associated with a predisposition to other hematopoietic malignancies or solid tumors. The *JAK2* gene seems again to play an important role in this predisposition. A haplotype, called 46/1, in *JAK2* introns confers a three-fold increase in the risk of developing a MPN.[153-156] The mechanism of this susceptibility is unknown. Furthermore, there is some evidence that mutations in *JAK2* coding sequence are also involved in familial forms of MPNs, especially in ET.[157,158] No JAK2V617F germ line mutations have been found, but other candidate germ line sequence variations in *JAK2* have been recently described[157,158] (Bellane-Chantelot, personal communication). These diseases may not be true ET, but rather a familial non-clonal thrombocytosis syndrome as already observed for the MPL S505N.[56] In other familial forms, there is evidence for true clonal MPNs with the presence of the acquired JAK2V617F mutation as in sporadic cases.[159] Germ line *TET2* mutations have only been found in one familial form.[160] The characterization of the molecular mechanisms in the other cases will be certainly an important breakthrough in the understanding of MPN pathogenesis.

Disease phenotype and mutations

The number of mutations found in MPNs remains quite intriguing because the hallmark of these disorders is a "simple" increased production of mature blood cells. There is clear evidence that a germ line mutation in a single gene may induce thrombocytosis, erythrocytosis, or neutrophilia. In addition, JAK2V617F murine models efficiently reproduce an ET- or PV-like disorder along with progression to myelofibrosis. There is no obvious need for mutations other than those affecting signaling pathways. In CML, the BCR-ABL fusion protein is sufficient by itself to recapitulate the entire disease.

There are two main complementary explanations for the complexity of MPN pathogenesis:

1) The classical MPNs are heterogeneous disorders and JAK2V617F mutation is associated with three phenotypes. However in mouse models, JAK2V617F induces either ET- or PV-like disorders progressing to myelofibrosis, but not PMF.[32-39] In a similar manner, MPLW515L induces an ET-like disorder progressing in a few months to myelofibrosis.[50,54] There is evidence that the signaling intensity determines the phenotype of the disorder in both the murine models and the human disease. A low signaling intensity induces an ET-like phenotype, whereas stronger signaling gives rise to PV and myelofibrosis. This is in line that mostly heterozygous JAK2V617F in ET and homozygous JAK2V617F in PV and secondary myelofibrosis are observed.[40] However, in addition to its strength, the type of signaling is also responsible for the phenotype. It has been suggested that PV, ET and myelofibrosis are associated, respectively, with a strong STAT5, STAT1 and STAT3 activation.[50,54,161,162] This difference in signaling may be due to numerous acquired or inherited determinants, such as gender, receptor expression level, silencing of SOCS, miR expression, and the presence of other mutations[163] Another possibility would be the type of HSC in which the mutation had occurred. The existence of myeloid-biased and lymphoid-biased HSCs, which respond differently to TGFβ1,[164] further suggests that mutations might lie in the myeloid-biased HSCs, which are increasing in number with age.[165,166] Furthermore, an Erythro/MK-biased HSC has been recently described that leads to a moderate thrombocytosis in reconstitution assays.[167] The occurrence of JAK2V617F in such HSC would likely induce an ET-like disorder. It has been also suggested that JAK2V617F may occur in a hemangioblast (a cell common to the hematopoietic and endothelial cell lineages in embryogenesis) inducing an activation of endothelial cells, which could be responsible for splanchnic thrombosis.[168]
   PMF greatly differs from ET and PV by the existence of variable myelodysplastic features and thus is not a pure MPN. In agreement with these hematological features, high frequencies of mutations in epigenetic regulators or in spliceosome genes are found in PMF with sometimes the coexistence of several different mutations. This correlates with animal models where TET2 or DNMT3A deficiency induces dysplastic features. It is presently unknown whether these mutations confer different disease progression in ET or PV, more specifically if the so-called pre-fibrotic myelofibrosis is an ET combining signaling molecules and epigenetic/spliceosome mutations. Thus, PMF could be considered as a disorder close to MPN/MDS, such as CMML, with similar pattern of mutations. The clinical

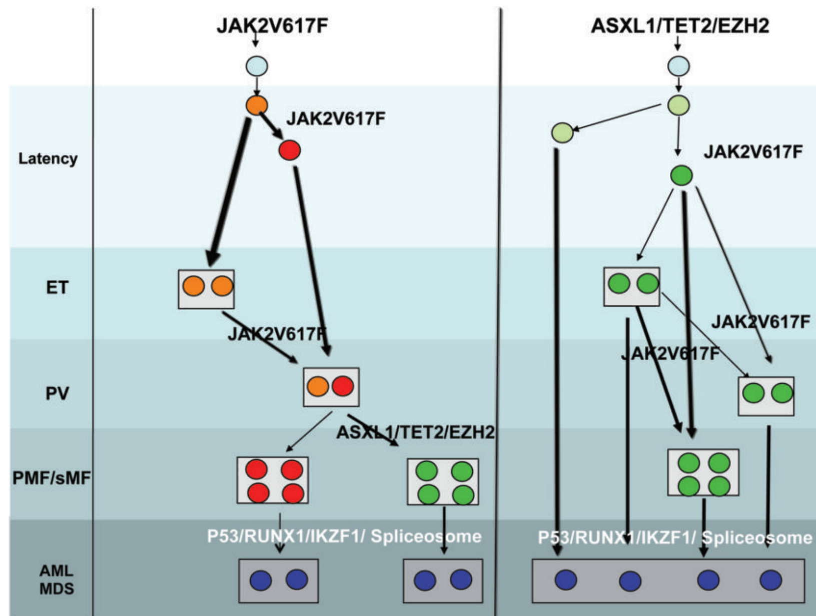

Figure 2. Models to explain the evolution of myeloproliferative neoplasms according to JAK2V617F or epigenetic regulator mutations as the initiating event.

phenotype will depend on the driver mutations (JAK2V617F in PMF and CBL, or RAS in CMML). In contrast, secondary myelofibrosis might be either the natural evolution of a PV or eventually an ET, or the transformation of the disease with the appearance of new mutations. This evolution might be facilitated by the gene instability induced by JAK2V617F.[23,169]

2) JAK2V617F induces a cytokine hypersensitivity that predominates on the proliferation of myeloid precursors during terminal differentiation. In contrast, the effects of JAK2V617F on HSC are modest. In PV and ET, amplification of the HSC and hematopoietic progenitor compartments is low, as well as the JAK2V617F allele burden in these cellular compartments.[41,170] This is confirmed in xenograft transplantation where the majority of HSC are JAK2 wild type.[171] Thus, in ET and PV, the clonal dominance takes place at late stages of differentiation.[41,172] In contrast in PMF, the majority of HSC and progenitors displays JAK2V617F.[170,171] This is in line with mutations in epigenetic regulators, such as TET2, which affect stem cell biology and may confer a clonal dominance at the level of early stages of differentiation.[93,101-104] In murine models, there are some controversial results about the effects of JAK2V61F on HSC. In one KI model, JAK2V617F was deleterious for HSC[39] whereas in the others, it did give a slight advantage to HSC.[34] There is recent additional evidence that JAK2V617F confers a competitive advantage to HSC. This modest effect on HSC may rely on the low expression of JAK2 in HSC.[170] Thus, JAK2V617F alone may be able to cause a MPN, but theoretically after a very long latency. In specific conditions like inflammation where JAK2V617F confers a resistance to TNFα[173] or during ageing where myeloid-biased HSC have a proliferative advantage,[165,166] JAK2V617F may endow the mutated HSC an increased advantage leading to faster development of the disease. Cooperation of JAK2V617F with other genetic events modifying HSC biology may also greatly facilitate clonal dominance. Moreover, the order of the mutations may not be important for disease development as these mutations occur in long life HSC. However, if the first mutation involves an epigenetic regulator, this may induce a preleukemic state that will progress either to MPD or MDS or AML depending on the secondary mutations. This may explain why a large fraction of JAK2V617F MPNs may transform in JAK2 wild type AML and also the presence in the same patients of two different MPNs (JAK2V617F, MPLW515L, JAK2 exon 2, or BCR-ABL).[174-176] Alternatively, some genetic instability due to either inherited or acquired presently unknown initiating event might be responsible for these observations.

Conclusions

In 2005, it was believed that the entire MPN pathogenesis would be explained by JAK2V617F. During the last years, an increasing number of mutations shared with other myeloid malignancies have been found in MPNs, suggesting that these disorders have a complex pathogenesis. In the near future, deep-sequencing techniques will certainly permit to identify new driver mutations presumably affecting signaling pathways, which are still elusive in about 35 to 40% of ET and PMF, and new cooperative mutations affecting other pathways. It will also facilitate the characterization of the combination of mutations present in the different MPNs. It will be especially important to determine if two main types of disorders co-exist in the MPN classification: those closely related to CML with a MPN-type initiating event, such as JAK2V617F, which accumulates other mutations during progression and those with complex combinations of mutations on different pathways on the model of MDS (Figure 2). These issues will have important consequences for understanding the pathogenesis of MPNs and the boundaries with MDS, and for the development of new therapies.

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