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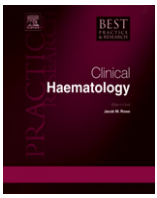

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Mechanisms of mutations in myeloproliferative neoplasms

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In recent years, a series of studies have provided genetic insight into the pathogenesis of myeloproliferative neoplasms (MPNs). It is now known that *JAK2V617F* mutations are present in 90% of patients with polycythaemia vera (PV), 60% of patients with essential thrombocytosis (ET) and 50% of patients with myelofibrosis (MF). Despite the high prevalence of *JAK2V617F* mutations in these three myeloid malignancies, several questions remain. For example, how does one mutation contribute to the pathogenesis of three clinically distinct diseases, and how do some patients develop these diseases in the absence of a *JAK2V617F* mutation? Single nucleotide polymorphisms at various loci and somatic mutations, such as those in *MPLW515L/K*, *TET2* and in exon 12 of *JAK2*, may also contribute to the pathogenesis of these MPNs. There are likely additional germline and somatic genetic factors important to the MPN phenotype. Additional studies of large MPN and control cohorts with new techniques will help identify these factors.

For every terminally differentiated myeloid cell, there is a clinical disorder that presents as a specific myeloproliferative neoplasm (Table 1). Patients who present with too many mast cells are classified as having systemic mastocytosis; too many red cells, polycythaemia vera (PV); and too many platelets, essential thrombocytosis (ET); and too many eosinophils, chronic eosinophilic leukaemia. In addition, there are several disorders that manifest as an excess of neutrophils and monocytes, including chronic myeloid leukaemia, myelofibrosis (MF), and chronic myelomonocytic leukaemia (CMML).

With the use of several different techniques, genetic insight has been gained into the pathogenesis of these diseases (Fig. 1). Chronic myeloid leukaemia is characterised by *BCR/ABL*, which can be targeted

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Table 1
Myeloproliferative disorders.

Type of myeloid cell	Disease/s	Activating mutation	Mutations being explored
Mast cell	Systemic mastocytosis	*KITD816V* *FIP1L1-PDGFRα*	TET2
Eosinophils	Chronic eosinophilic leukemia	*FIP1L1-PDGFRα*	
Neutrophils	Chronic myeloid leukemia	*BCR-ABL*	
Monocytes	Chronic myelomonocytic leukemia	*TEL-PDGFRβ* *BCR-PDGFRα* *TEL-JAK2* Other fusion TKs	TET2, ASXL1
	Primary myelofibrosis	*JAK2V617F, MPLW515L*	*TET2*
Erythrocytes	Polycythemia vera	*JAK2V617F*	*TET2*
Platelets	Essential thrombocytosis	*JAK2V617F, MPLW515L*	*TET2*

with imatinib mesylate, a tyrosine kinase inhibitor [1]. Systemic mastocytosis is characterised by activating mutations in *KITD816V* and *FIP1L1–PDGFRα*. The *KITD816V* mutation is insensitive to imatinib, necessitating the use of second-generation tyrosine kinase inhibitors [2]. Other work has implicated *FIP1L1-PDGFRα* in the pathogenesis of chronic eosinophilic leukaemia, and patients with the mutation are also sensitive to imatinib [3,4]. CMML is often, but not always, characterised in different patients by the presence of one of a set of activated fusion tyrosine kinases, including TEL-PDGFRβ, BCR-PDGFRα, TEL-JAK2 and others.

Activating mutations in *JAK2*

The genetic evidence suggesting that tyrosine kinase acquisition was a common pathogenetic event in MPNs led to the hypothesis that PV, ET and MF were also driven by activated tyrosine kinases. Samples from patients with these diseases were collected, and the relevant domains of all 90 tyrosine kinases were sequenced. This tyrosine kinome screening approach, and several other alternative approaches, led to the discovery of activating mutations in *JAK2* in 90% of patients with PV, in 60% of patients with ET and in 50% of patients with MF [5–8]. The most common *JAK2* mutation substitutes

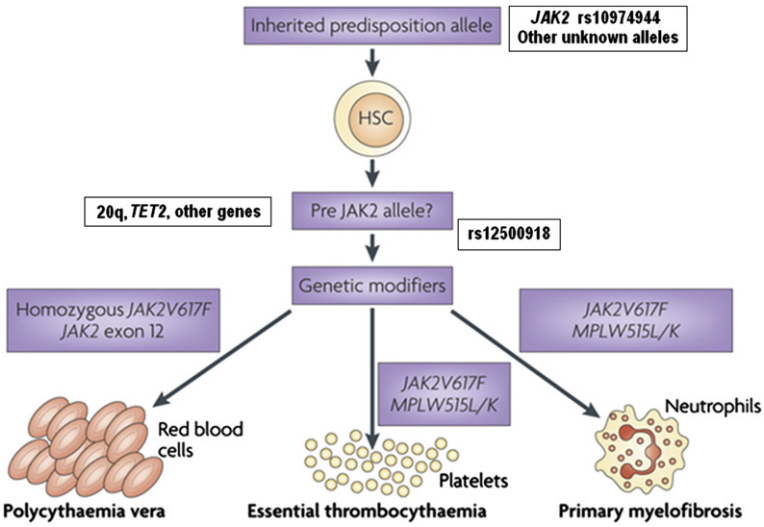

Fig. 1. Working model of MPN pathogenesis [17]. This current working model of MPN pathogenesis shows acquired and inherited alleles in conjunction with genetic modifiers. © 2007 Nature Publishing Group, from Levine et al, Nature Reviews Cancer.

valine for phenylalanine at the 617 position of the gene. Although four other residues can activate *JAK2* by substitution [9], none has been observed in humans, suggesting a special role for the V/F substitution at codon 617. *JAK2* mutations are present in blood cells from patients with these MPNs, but are not present in matched normal DNA, consistent with their being acquired as somatic disease alleles. This observation highlights the importance of using non-haematopoietic DNA from patients to determine whether candidate mutations are inherited or acquired. Moreover, the presence of heterozygous *JAK2V617F* mutations in some patients and homozygous *JAK2* mutations in other patients makes it important to determine the differences between homozygous mutants and heterozygous mutants. A subset of patients, particularly those with ET, is exclusively heterozygous for the *JAK2* mutation, and another subset of patients, particularly those with PV, has a significant number of homozygous haematopoietic mutations. This suggests that a single activating mutation in *JAK2* is not saturating for activation of the myeloproliferative pathway.

When *JAK2V617F* is expressed in murine haematopoietic stem cells, the *in vivo* phenotype is rapid, fully penetrant PV [10]. Mice with the mutation also demonstrate strain-dependent leucocytosis, hepatosplenomegaly and reticulin fibrosis, which are some of the features of primary MF. However, the absence of thrombocytosis in these mice is not understood, and the model does not produce an ET phenotype. This may be due to the fact that the model does not accurately assess the effects of *JAK2V617F* gene dosage. The expression of different amounts of the mutated JAK2 protein might lead to different phenotypes *in vivo*, as has been shown more recently with transgenic models expressing *JAK2V617F* at much lower levels, which results in thrombocytosis [11].

Despite the number of very important observations on *JAK2* mutations, some basic unanswered questions remain. The structural and functional consequences of the V617F substitution are unknown, and no one has been able to successfully determine the exact structure of the pseudokinase domain of JAK2 with or without the V617F substitution. The effect of *JAK2V617F* gene dosage on signalling and on phenotype, as mentioned above, is not understood. Moreover, it is not clear how a single point mutation can contribute to the pathogenesis of three related but clinically distinct diseases. The role of targeted therapy against *JAK2V617F* has yet to be fully determined, and trials of JAK2 inhibitors are ongoing in MPNs. Finally, the aetiologies of *JAK2V617F*-negative PV, ET and MF have yet to be completely explained.

JAK2V617F-*negative MPNs*

Almost all PV patients with *JAK2V617F*-negative disease have been found to have activating mutations in a different domain within *JAK2* involving residues 538–543 within exon 12 [12]. Fifteen different acquired mutations have been described in the SH2 domain of *JAK2* to date. These mutations can be homozygous or heterozygous, and all are exclusively associated with PV. Patients with somatic activating missense/deletion mutations in exon 12 involving residues 538–543 most commonly present with isolated erythrocytosis, but not the pan-myeloid expansion seen with *JAK2V617F*-positive PV. Expression of the exon 12 *JAK2* mutation *in vivo* results in a polycythemic phenotype similar to that seen with *JAK2V617F*.

In another study, samples from *JAK2V617F*-negative patients with ET or MF were examined for mutations in the cytokine receptors that bind JAK2 [13,14]. Many of the samples had a tryptophan-to-leucine substitution at codon 515 on the thrombopoietin receptor (*MPL*). *MPLW515L/K* is an acquired somatic mutation present in blood cells but not in germlines. The mutation is present in 10% of patients with primary MF and in 5–8% of patients with ET. *In vitro* effects of *MPLW515L/K* are similar to effects of *JAK2V617F*, and cells transformed with *MPLW515L/K* are sensitive to JAK2 inhibitors. However, *in vivo MPLW515L/K* expression results in massive thrombocytosis and megakaryocytic hyperplasia. This suggests that *MPLW515L/K* alleles increase proliferation, but alleles of *JAK2* and *MPL* affect the differentiation of stem cells in different ways: *MPL* mutants tend towards the megakaryocyte lineage, while *JAK2* mutant stem cells tend towards an erythroid lineage. It is therefore important to understand what pathways are activated by the different mutations in the JAK–STAT pathway.

The role of JAK–STAT signalling in *JAK2*/*MPL*-negative MPNs is unknown. Sequence analysis of 48 *JAK2*/*MPL*-negative MPN patients did not reveal mutations in *JAK1*, *JAK2*, *JAK3*, *TYK2*, *MPL*, *EPOR*, *GCSFR*,

LNK, *SH2BB*, *STAT5A*, *STAT5B* or *STAT3*. Although this focussed mutational analysis of the JAK–STAT pathway was negative, knowledge of this signalling pathway may be incomplete. New sequencing technologies will allow for whole genome resequencing, which may aid in the identification of mutations outside the JAK–STAT pathway in *JAK2*/*MPL*-negative MPNs.

One mutation, three diseases

Three possibilities exist for how the *JAK2V617F* mutations can cause three distinct clinical disorders. There may be *JAK2*-dependent effects. A homozygous cell may be more likely to develop an erythroid phenotype than a heterozygous cell, which may be more likely to develop into ET. The *JAK2* expression level may also be different in the distinct diseases. There may also be *JAK2*-independent effects such as second somatic mutations or epigenetic lesions that co-operate with *JAK2* in the pathogenesis of these disorders. Finally, germline genetic variation may also be implicated in the differential pathogenesis of the three diseases.

In a genome-wide single nucleotide polymorphism (SNP) analysis, 250 000 different loci were studied in samples from 207 MPN patients. The study aimed to determine whether there are loci that segregate with specific MPN diagnoses or with MPN patients compared with controls. This enabled researchers to distinguish haplotypes of those with a PV phenotype versus those with an ET phenotype. Four loci, rs12500918, rs1524395, rs10974944 and rs2279784, proved to be statistically significant. Three of the loci were novel, but rs10974944 is located in a 178-kbp haplotype block of the *JAK2* gene. In addition, the rs10974944 germline haplotype occurs more commonly in PV than ET. Furthermore, the *JAK2* SNP is significantly more common in MPN patients than in healthy controls, suggesting that the rs10974944 variant may be a predisposition locus in the germline that leads to the somatic acquisition of *JAK2* mutations. Therefore, rs10974944 does not necessarily segregate PV from ET, but might segregate MPNs from healthy controls [18,19, 20].

To determine whether rs10974944 predisposed patients to the development of MPN, germline tissue from MPN patients was compared with clonal tissue. Results showed that the *JAK2* SNP was present in the germline and retained its statistical significance as a predisposition allele. Furthermore, the haplotype was only significantly enriched in *JAK2*-positive MPN patients, suggesting that the haplotype predisposes patients to acquisition of the *JAK2* mutation and not to MPNs as a whole. The rs10974944 SNP was also not enriched in populations of patients with acute myeloid leukaemia or myelodysplastic syndromes (MDS). In a study of 42 patients with the rs10974944 and a *JAK2V617F* mutation, *JAK2V617F* was preferentially acquired on the same strand of DNA as the inherited allele in 38 patients. There were no coding variants or untranslated region variants in *cis* with the risk allele. These data demonstrate that germline variation at the *JAK2* locus influences the risk of developing MPNs by increasing the likelihood of acquiring somatic *JAK2* mutations.

Somatic mutations

Clonality data suggest there is a larger population of clonal cells than *JAK2*-mutated cells. There are a small number of patients with cytogenetic abnormalities in a larger proportion of cells than the number of cells that are *JAK2* mutant. These co-operating somatic lesions may even precede the acquisition of *JAK2*, which may have relevance to JAK2 inhibitor efficacy in the different MPNs, and the mutational context is likely to be critical. In a recent study, a somatic mutation in *TET2* was found in 15–20% of patients with *JAK2*-mutant MPNs and MDS [15]. The *TET2* mutation precedes the acquisition of *JAK2* in some patients, but several issues still need to be clarified. The function of the mutant *TET2* gene and its genetic relevance are unclear; its clinical and genetic correlation in MPN and MDS is unknown; and it is not known whether epigenetic inactivation of *TET2* or mutations in *TET1* or *TET3* contribute to MPN pathogenesis.

To determine the relevance of *TET2* mutations in a broader context of myeloid malignancies, we have performed high-throughput resequencing of *TET2* in 775 patients with myeloid malignancies, including 513 samples with matched normal DNA [16]. Most of the *TET2* mutations are frameshift mutations or truncation mutations, and all are somatic, not germline, mutations. Mutant *TET2* has been

found in every type of myeloid malignancy assessed to date, including 10% of MPN patients, 40% of CMML patients and 12% of acute myeloid leukaemia (AML) patients. The *TET2* mutation has also been found in every cytogenetic subset of AML, and *TET2* mutations occur in patients with and without known oncogenic disease alleles, such as *JAK2*, *FLT3* and *RAS*. There may also be additional mutations in *TET2*-regulated pathways in myeloid malignancies. Furthermore, *TET2* does not seem to correlate with any clinical, cytogenetic, genetic or prognostic parameter, so it likely does not stratify a unique subset of patients. The relevance of *TET2* mutation to therapeutic responses to novel or known therapeutic agents has yet to be determined.

Conclusions

The current working model of MPN pathogenesis (Fig. 1) is composed of acquired and inherited alleles in conjunction with genetic modifiers. Inherited alleles, such as *JAK2* andrs10974944, predispose people to the acquisition of the *JAK2V617F* allele. There are also pre-*JAK2* mutation alleles, such as *TET2*, a likely tumour suppressor gene on 20q, and others that may precede the acquisition of *JAK2V617F* alleles. Finally, a number of inherited and somatic genetic events likely dictate what disease will develop in a germline or acquired somatic context. Additional studies using candidate-gene and whole-genome studies will undoubtedly identify additional genetic factors that contribute to the pathogenesis of these myeloid neoplasms.

Conflict of interest statement

My only conflict is that I receive research funding from Novartis and Astra Zeneca. R.L.L. is an Early Career Award recipient of the Howard Hughes Medical Institute and is the Geoffrey Beene Junior Chair at Memorial Sloan Kettering Cancer Center.

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