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Pathogenesis of myeloproliferative neoplasms

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Major progress has been recently made in understanding the molecular pathogenesis of myeloproliferative neoplasms (MPN). Mutations in one of four genes—*JAK2*, *MPL*, *CALR*, and *CSF3R*—can be found in the vast majority of patients with MPN and represent driver mutations that can induce the MPN phenotype. Hyperactive JAK/STAT signaling appears to be the common denominator of MPN, even in patients with *CALR* mutations and the so-called "triple-negative" MPN, where the driver gene mutation is still unknown. Mutations in epigenetic regulators, transcription factors, and signaling components modify the course of the disease and can contribute to disease initiation and/or progression. The central role of JAK2 in MPN allowed development of small molecular inhibitors that are in clinical use and are active in almost all patients with MPN. Advances in understanding the mechanism of JAK2 activation open new perspectives of developing the next generation of inhibitors that will be selective for the mutated forms of JAK2.

Myeloproliferative neoplasms (MPN) are a group of diseases characterized by increased proliferation of erythroid, megakaryocytic, or granulocytic cells. The concept to group several clinical entities under this umbrella goes back to William Dameshek, who in 1951 recognized that these disorders are caused by hyperproliferation of multiple hematopoietic lineages in the bone marrow that proliferate "as a unit" [1]. Dameshek proposed the term *myeloproliferative disorders* to indicate that these entities may represent a continuum of related syndromes. He also proposed that the proliferative activity could be perhaps due "to a hitherto undiscovered stimulus" [1]. However, the finding that bone marrow and peripheral blood cells from MPN patients can produce erythroid colonies in vitro in the absence of added growth factors indicated the cell autonomous nature of these diseases [2], and the clonal origin of peripheral blood cells of MPN patients was later proven by analyzing X-chromosome inactivation patterns [3]. The classification proposed by the World Health Organization (WHO) is currently most widely used and defines eight entities (Table 1) [4]. This classification will soon be revised and modified to incorporate recent advances in the molecular characterization of these diseases [5]. In this review, we will focus primarily on the so-called Philadelphia chromosome–negative or *BCR-ABL1*-negative MPN, that is, polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and chronic neutrophilic leukemia (CNL).

Driver mutations that cause myeloproliferation in Ph-negative MPN

Only 10 years ago, essentially nothing was known about the molecular pathogenesis of MPN. The first gene mutation described in 2005, *JAK2*-V617F, turned out to be the most important and most frequently recurring somatic mutation in MPN [6–9]. The frequency of *JAK2*-V617F is around 95% in PV and between 50% and 60% in ET and PMF (Fig. 1). Expression of *JAK2*-V617F in cell lines abrogates their growth factor dependence, and retroviral expression of *JAK2*-V617F in hematopoietic cells in mouse models leads to an MPN phenotype resembling the human PV [6,10–13]. These data indicated that the *JAK2*-V617F mutation results in a gain of function. Somatic mutations in other positions in *JAK2* have been subsequently found in PV (*JAK2* exon 12 mutations) [14] and B-cell acute lymphocytic leukemias (*JAK2* exon 16 mutations) [15–19]. More recently, a number of *JAK2* germline mutations

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Table 1. 2008 classification of myeloproliferative neoplasms by the World Health Organisation [4]

1	Chronic myelogenous leukemia, *BCR-ABL1* positive (CML)
2	Chronic neutrophilic leukemia (CNL)
3	Polycythemia vera (PV)
4	Primary myelofibrosis (PMF)
5	Essential thrombocythemia (ET)
6	Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS)
7	Mastocytosis
8	Myeloproliferative neoplasm, unclassifiable (MPN-U)

have been described in familial syndromes, most of them associated with a thrombocytosis phenotype [20–22].

Activating mutations in the thrombopoietin (Tpo) receptor, *MPL*, can be found either as germline mutations in rare cases of familial thrombocytosis (*MPL*-S505N) [23] or as somatic mutations that occur in 3%–8% of patients with PMF or ET (*MPL*-W515) [24,25]. The mechanism of how these missense mutations result in Tpo-independent signaling by the mutant Mpl protein involves alterations in the geometry of Mpl dimers and the attached Jak2 proteins [26]. The tryptophan residue (W) in position 515 at the intracellular juxtamembrane boundary normally inhibits dimerization of the Mpl transmembrane helix and thereby prevents receptor self-activation [27]. Replacing W515 with another amino acid, for example, leucine, lysine, or arginine, leads to loss of this inhibition and results in a constitutively active Mpl. The expression levels of Jak2 and Mpl proteins increase during maturation and constitute another level of control that is involved in the fine-tuning of megakaryopoiesis [28]. Activating mutations in the granulocyte colony-stimulating factor (G-CSF) receptor, CSF3R, that also signals through Jak2 were first found in familial neutrophilia [29] and later in sporadic CNL [30,31]. They cluster in the extracellular domain of CSF3R. In some cases, activating mutations can coexist with truncating mutations in the cytoplasmic domain of CSF3R found in severe congenital neutropenia [32]. Although CNL is a rare disease and was sometimes difficult to distinguish from cases of atypical chronic myeloid leukemia (CML), the presence of recurrent *CSF3R* mutations will now make it simple to define CNL as an entity related to PV, ET, and PMF, but with a specific phenotype and mutational profile. Thus, the theme emerging from these studies is that Ph-negative MPN is frequently caused by mutations in JAK2 or in cytokine receptors that depend on JAK2 for their signaling.

A major gap in the mutational profile of ET and PMF was recently filled with the discovery of somatic mutations in calreticulin (*CALR*) that occur in 20% to 35% of patients with ET or PMF [33,34]. The *CALR* and *JAK2* mutations are mutually exclusive in patients with MPN, although rare exceptions can occur [35]. These *CALR* mutations result in a frameshift into an alternative reading frame that alters the C-terminal sequence of the protein [33,34]. The mutant CALR protein lacks the C-terminal endoplasmic reticulum retention signal (KDEL) and most likely has impaired Ca^{2+} binding function. Although the mechanisms of how *CALR* mutations cause MPN has not yet been resolved [36], they also ultimately lead to hyperactivity of the JAK2/Stat signaling pathway in megakaryocytic and granulocytic progenitor and precursor cells [33,37]. Interestingly, patients with *CALR* mutations and unmutated *JAK2* also respond to JAK2 inhibitors [38,39]. Finally, MPN patients who do not carry mutations in any of the aforementioned genes (so-called "triple-negative" MPN cases) also appear to have hyperactive JAK2 signaling [37]. Therefore, it seems appropriate to consider the Ph-negative MPN as diseases driven by hyperactive Jak2/Stat signaling.

Other gene mutations frequently occurring in MPN

In addition to the "phenotypic driver mutations" that are directly linked to hyperproliferation of hematopoietic cells (Fig. 1), there is a growing list of somatic mutations

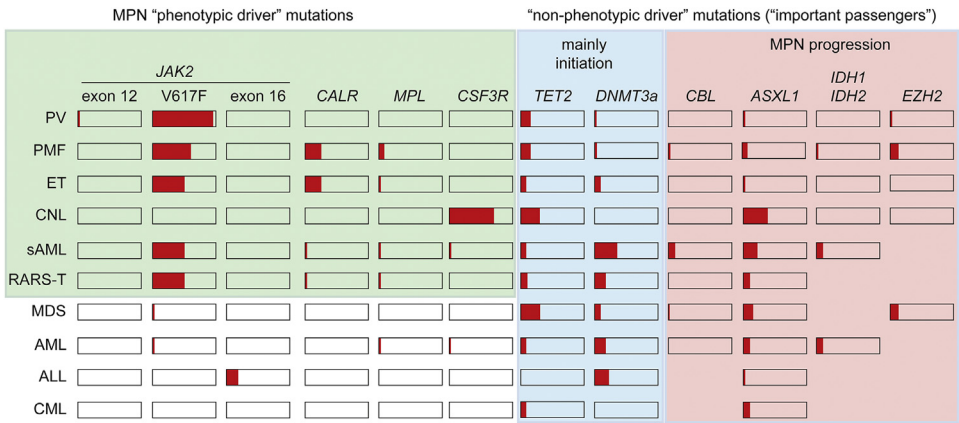

Figure 1. Frequency and distribution of acquired gene mutations in hematologic malignancies. The *red portions* of the horizontal bars indicate the approximate frequencies of the mutations in the different disease entities. ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; CML = chronic myeloid leukemia; CNL = chronic neutrophilic leukemia; ET = essential thrombocythemia; MDS = myelodysplastic syndromes; MPN = myeloproliferative neoplasms; PMF = primary myelofibrosis; PV = polycythemia vera; RARS-T = refractory anemia with ring sideroblasts and thrombocytosis; sAML = secondary acute myeloid leukemia.