Review

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Molecular basis and clonal evolution of myeloproliferative neoplasms

Abstract: Myeloproliferative neoplasms (MPNs) represent a group of diseases that affect the myeloid lineage, characterized by the presence of an excess of terminally differentiated myeloid cells. Defects causing clonal hematopoiesis are a key factor in the emergence of these diseases. Throughout the years, a number of causative defects have been identified, predominantly affecting cytokine signaling and gene expression regulation. This review aims to provide an update on the current status of the MPN field in relation to identification of molecular defects involved in the disease and its clonal evolution.

Keywords: JAK2; leukemia; myeloproliferative neoplasms; oncogene; tumor suppressor.

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Introduction

Myeloproliferative neoplasms (MPNs) constitute a heterogeneous group of diseases in which there is an increased production of clonal myeloid cells and frequent evolution to acute myeloid leukemia (AML) [1]. The disruption of hematopoiesis is a hallmark feature of MPNs. Hematopoiesis is a dynamic and highly regulated process in which differentiation and proliferation of hematopoietic cells are tightly coupled. Acquired mutations (point mutations or chromosomal aberrations) may disrupt this process, resulting in a wide range of defects affecting the myeloid lineages. The majority of somatic mutations that accumulate during the division of pluripotent hematopoietic stem cells often have no phenotypic effect, although certain mutations seem to provide a proliferative advantage. As a consequence, a stem cell carrying such an advantageous mutation enters the cell cycle more often and establishes a clone that expands over time, resulting in monoclonal hematopoiesis. Establishment of monoclonal hematopoiesis is a characteristic feature of all myeloid malignancies. One of the frequent phenotypic outcomes of clonal hematopoiesis in humans are MPNs. The current classification of MPN by the World Health Organization includes nine disease entities; however, only the classical BCR-ABL-negative MPNs are the subject of this overview [1]. The classical MPN include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). PV occurs as a result of the overproduction of red blood cells; ET results from the overproduction of platelets; and PMF is characterized by the accumulation of excess fibrotic tissue in the bone marrow.

Over the past decade, genomics has enabled the discovery of a diverse range of genes in MPN pathogenesis, indicating that many different cellular pathways are responsible for the clonal evolution of stem cells in the disease. The somatic mutations associated with MPN identified so far fall into two major functional categories: (1) mutations affecting cytokine receptor signaling and (2) mutations associated with regulation of gene expression (Table 1). This review will discuss the progress made to date in elucidating the molecular basis of the disease and its clonal evolution. In addition, we will also address the contribution of various lesions to disease progression and their use as prognostic markers.

Mutations affecting the JAK-STAT signaling pathway

Cytokine signaling plays a major role in hematopoiesis and is essential for the maintenance of hematopoietic stem cells and control of proliferation and differentiation of committed progenitor cells. A fine balance between
cytokine production and signaling response is required for the generation and maintenance of blood cell homeostasis. Because cytokines such as erythropoietin (EPO) and thrombopoietin (THPO) directly influence erythroid and megakaryocytic progenitor cell numbers, it is no surprise that many of the genes that mutated in MPNs target this pathway. Proteins involved in the stabilization of signaling components, tyrosine kinases, and signaling adaptors are just a few examples of genes that have been implicated in the disease.

In 2005, a mutation in the JAK2 gene was identified by several groups that could be observed in over 90% of PV cases and approximately 50%–60% of ET and PMF patients [2–5]. The JAK2 protein plays a pivotal role in cytokine signaling in hematopoiesis, mediating signals from a wide range of cytokines and growth factors. This particular mutation, a valine-to-phenylalanine substitution, occurs in exon 14 of the gene, resulting in the constitutive activation of the downstream signaling pathways [6]. Under normal conditions, it is activated upon the dimerization or oligomerization of cytokine receptors such as erythropoietin (EPO), thrombopoietin (THPO), and granulocyte colony-stimulating factor receptor (GCSFR) in the presence of a ligand [7]. However, the V617F mutation renders the JAK2 protein constitutively active in the absence of a corresponding stimulus. Mutations in other parts of the gene, including exon 12, have also been observed in a subgroup of MPN patients [8, 9]. The identification of such a frequently mutated gene prompted further investigation into its role in the development of the disease. This was achieved using various approaches including murine models to recapitulate the myeloproliferative phenotype seen in humans. To date, several groups have reported the generation of murine models of the JAK2-V617F mutation; however, the myeloproliferative phenotype slightly differs between the various models. These models include bone marrow-transplanted, transgenic, and targeted knockout mice [10–14]. As the myeloproliferative phenotype in some mouse models showed strain-specific differences, it is speculated that germline genetic factors may contribute to the development of the disease in addition to the acquired JAK2-V617F mutation. Nevertheless, all the studies concluded that the JAK2 mutation is sufficient to produce a myeloproliferative phenotype.

Following the discovery of JAK2 mutations, attention was drawn to other members of the JAK-STAT signaling pathway. This pathway can be activated through many different growth factor/receptor interactions including the myeloproliferative leukemia virus (MPL) oncogene. Mutations in this receptor were identified in a few ET and PMF cases. In particular, exon 10 of MPL is a hotspot for mutations in MPN, mostly occurring in patients who are JAK2-V617F-negative presenting with myelofibrosis and, to a lesser extent, ET [15]. MPL-W515L and W515K mutations in exon 10 were found to be a gain-of-function mutation, leading to THPO-independent growth when investigated in a variety of cell lines and mouse bone marrow [15]. Throughout the years, a few more mutations have been identified in exon 10 [16]. Functionally, MPL mutations target the cytoplasmic juxtamembrane or transmembrane regions of the receptor, which is responsible for preventing random activation of the receptor in the absence of THPO [17]. Overall, this gene accounts for approximately 15% of JAK2-V617F-negative MPNs and is known to appear early in the disease, indicating its role as a clonal and disease driver [18].

### Mutations targeting the negative regulators of the JAK-STAT pathway

Another important aspect of cytokine signaling is the negative regulation of its components. For instance, JAK2 has a number of negative regulators including LNK, SOCS family proteins, and CBL. The LNK gene, also known as SH2B3, is another target of mutations in MPN and is a key negative regulator of the JAK-STAT signaling pathway. It encodes an adaptor protein that, upon cytokine stimulation, binds to JAK2, resulting in the inhibition of further downstream signaling. Its role in MPNs first came to light through the observation of corresponding knockout mice that developed a strong MPN phenotype with clinical features such as splenomegaly and severe defects in hematopoiesis [19, 20]. It has also been shown to bind to MPL, thus affecting both JAK2 and MPL signaling [21, 22].
Mutations in this particular gene are somewhat rare and have only been identified in a few JAK2-V617F-negative patients; however, there has been some evidence of a link with progression to AML [23–25]. Another negative regulator of cytokine signaling that has been identified as a possible culprit in the disease is SOCS2. Lesions targeting a variety of SOCS proteins (SOCS1 and SOCS3) have been identified in a number of MPN patients [26–28]. These proteins inhibit the activity of JAK by binding to JAK itself or to receptors upstream of JAK2 signaling. Mutations in SOCS genes are somewhat rare in MPNs; however, many studies have reported the disruption of such genes in MPNs via the hypermethylation or deletion of the gene itself [27, 28].

Mutations in CBL have been reported in a wide range of myeloid malignancies, ranging from AML to MDS [29–34]. With regard to MPNs, mutations in this gene are primarily found in PMF patients and rarely in ET or PV patients [33]. CBL itself functions as an E3 ubiquitin ligase and plays an important role in the regulation of cytokine signaling. Using its ubiquitinating function, it can target certain proteins to proteasomal degradation, thereby acting as a negative regulator of the cytokine receptor signaling cascade, with targets such as JAK2, GRB2, and EPOR [35, 36]. Mutations in the gene seem to impair its ubiquitin ligase function and often result in a dominant negative effect [35].

NF1 and NRAS constitute parts of the MAPK signaling pathway, one of the primary downstream targets of JAK2. Recent studies have indicated that members of this pathway, such as NF1 and NRAS, are often mutated in MPNs, contributing to the myeloproliferative phenotype [37, 38]. Mutations in NRAS seem to result in the constitutive activation of the protein, thus affecting the expression of the JAK2 target genes. NF1 is a well-known negative regulator of RAS, enhancing its intrinsic GTPase activity via the hydrolyzation of GTP to GDP. Deletions of the NF1 tumor suppressor gene have been observed in many cohorts of patients, particularly those in chronic phase, whereas NRAS mutations seem to primarily affect patients that have transformed to AML [38].

Mutations affecting the epigenetic landscape and gene expression regulation

Dysregulation of gene expression is a common feature of MPNs, and this occurs mainly via the disruption of transcription factors themselves or through an alternative route involving epigenetic factors. The recent discovery of an epigenetic role of JAK2 indicates that this cellular pathway may be important in the development of the disease [39]. A recent study has shown that in AML, patients can be segregated into 16 subtypes, depending on their DNA methylation profiles, which in turn correspond to subtypes of AML as specified by the current classification system [40]. High-throughput sequencing of many cohorts of patients has identified a plethora of aberrations in genes involved in epigenetic mechanisms as well as transcription factors. We expect a similar complexity in various MPN subtypes.

Transcription factor mutations

Transcription factors are the essential components of gene expression regulation. Mutated or deleted transcription factors first reported in MPN include IKZF1, CUX1, and EZH2 [26, 41, 42]. Notably, these three genes are all located on chromosome 7, providing a plausible explanation as to why the monosomy of chromosome 7 is quite a severe cytogenetic lesion in MPN. Additional high-resolution cytogenetic studies have identified a number of transcription factors deleted in MPN such as ETV6, FOXP1, RUNX1, and CUX2 [26]. However, these deletions occur at a relatively low frequency in MPN, and research is still ongoing into how exactly these deletions exert an effect on hematopoietic stem cells. Interestingly, some of these transcription factors have been associated with progression to AML such as CUX1 and IKZF1, although the prognostic value of these deletions is yet to be determined [26].

Epigenetic factor mutations

Epigenetic factors also play a pivotal role in regulating gene expression. This regulation can be achieved through the methylation/acetylation of cytosines or histones. In fact, CpG hypermethylation is one of the most common ways of achieving transcriptional repression. One example of an epigenetic gene affected by various mutations in MPN is TET2. TET2 encodes an enzyme that catalyses the switch of 5-methyl cytosine into hydroxymethylated cytosine, requiring Fe(II) and α-ketoglutarate [43]. The sequencing of patient samples has revealed many mutations targeting the TET2 gene, indicating a possible role of the protein in disease development [44–46]. It is speculated that TET2 affects gene expression through its role in DNA demethylation. TET2-deficient mice often present with reduced levels of methylated cytosines in their genome alongside defects.
in the hematopoietic department [47–49]. Both mutations and deletions of the TET2 gene have been frequently observed in patients, with mutations occurring in 13% and deletions in approximately 3% [27, 44, 45]. A recent finding has shed more light on the function of TET2, where somatic mutations were found in normal elderly individuals presenting with clonal hematopoiesis [50]. This discovery, alongside the mouse studies, supports the notion that TET2 enhances self-renewal and clonal expansion in stem cells. However, further studies are required to fully investigate its precise role in malignant transformation.

**DNMT3A** is another interesting gene affected in MPN because it encodes a DNA de novo methyl transferase, which enables the transfer of a methyl group to cytosines in CpG dinucleotides. Mutations in this gene have been reported in approximately 10% of MPN patients and also in other myeloid malignancies, often accompanied by other lesions in genes such as **IDH1/2**, **TET2**, **ASXL1**, or **JAK2** [51, 52]. Debate is ongoing in relation to whether this gene acts as a tumor suppressor or as an oncogene because evidence exists for both a gain-of-function role and a loss-of-function role [51, 52].

## Mutations in histone modifiers and splicing factors

Aberrations affecting genes involved in histone modifications have also been described in MPNs such as the components of the polycomb repressor complex (PRC2). This complex functions by modifying certain histones, leading to repressive effects on gene expression, which in turn often affect processes such as development and cell proliferation [53]. The additional sex comb-like 1 gene (**ASXL1**) encodes a gene involved in the polycomb repressor complex, affecting the expression of various HOX genes [54]. This particular protein has been found to be targeted by mutations in up to 7.8% of MPN cases [55]. Other studies have shown that it is more frequent in PMF and disease progression, indicating a possible role for the gene in the development of the phenotype and transformation [56, 57]. Other polycomb protein members implicated in the disease are **EZH2**, **JARID2**, **AEBP2**, **EED**, and **SUZ12**, which have been found to be mutated or deleted in patients presenting with both MPN and MDS [41, 58–60].

Lastly, splicing factors play an important role in the mRNA-processing pathway; thus, aberrant splicing can have a major effect on the expression of a gene. Mutations in many splicing factors have been discovered in a wide range of myeloid malignancies and seem to cluster within MDS patients [61]. However, in MPNs, up to 9A% patients present with lesions affecting the splicing machinery [61]. The identified genes include **SF3B1**, **SRSF2**, **U2AF1**, and many others. In particular, SF3B1 mutations seem to occur in about 4%–6.5% of patients with PMF and 3% of patients with ET; however, there is no clinical association. One of the more interesting of these splicing factors identified is **SRSF2**, which has been shown to be associated with disease progression in MPN [62].

## Clonal evolution of MPN and lesions associated with leukemic transformation

Due to the sheer complexity of the disease, further research has been conducted into determining the genes involved in the initiation and progression of the disease, which are of great interest to both researchers and clinicians. **JAK2-V617F** and **MPL** mutations have already been shown to be sufficient to drive the myeloproliferative phenotype in mice, and the acquisitions of other cytogenetic lesions often follow these particular mutations. Several studies have reported the appearance of a **TET2** mutation before **JAK2**, indicating a potential role for the gene in the evolution of the disease. Lesions in other genes such as **NF1** and **CBL** tend to appear later, usually toward the leukemic phase. It is important to note that almost one third of MPN cases do not possess any detectable cytogenetic lesion or **JAK2/MPL** mutations. Current research is aiming to cover this ‘gap’ in MPN biology through the use of ever-improving sequencing technologies. Despite this ‘gap’, one conclusion that can be made is that the acquisition of all these cytogenetic lesions after the appearance of **JAK2-V617F** has no real pattern and appears to be acquired randomly in the context of disease evolution, with a few showing an association with disease progression to AML (Figure 1).

MPNs can be divided into at least three different stages: chronic phase, accelerated phase, and the leukemic phase. The chronic phase is characterized by a stable disease. The accelerated phase is a stage where a certain amount of cytopenia is present and secondary myelofibrosis or a steady increase of blasts can be observed. Lastly, the leukemic phase is the stage that is clinically defined by a blast frequency of over 20% in the bone marrow and with a variable degree of cytopenia [1]. In general, the risk of leukemic transformation for an individual diagnosed with MPN is 7% [9]. During recent years, many different genes have been implicated in MPN; however, not all affect the risk of progression of the disease. One of the
most prominent genes in MPNs, \textit{JAK2}, has already been shown to affect the risk of disease progression depending on the allele burden in the individual [63]. \textit{TP53} is a well-known tumor suppressor gene implicated in many forms of cancer. However, in MPNs, mutations are rarely observed in the chronic phase and are often observed in the leukemic phase, at a frequency of approximately 20\% [26, 64]. Therefore, acquisition of the \textit{TP53} mutation may stimulate the transformation of the chronic phase to the leukemic phase. Interestingly, de novo AML patients present with very low mutation rates of \textit{TP53}, indicating that some other factors may be involved, such as the type of therapy used to manage this particular MPN [65, 66]. Cytogenetic aberrations are another interesting aspect of disease progression because patients with post-MPN AML often possess a much more complex karyotype than those with the chronic phase [67]. Monosomy 7 is an interesting lesion because it has a poor prognosis and targets many genes including \textit{IKZF1}, \textit{CUX1}, and \textit{EZH2} [26]. Secondary AML shares many defects with de novo AML such as \textit{IDH1/2}, \textit{FLT3}, \textit{DNMT3A}, \textit{NPM1}, and \textit{RUNX1} [26, 68–75]. This indicates that these genes may be involved in the overall leukomogenesis pathway but not specifically for transformation in MPNs. Deletions of 7q have also been found to be overrepresented in post-MPN AML and seem to target the \textit{CUX1} transcription factor, which is involved in the cell cycle and general hematopoiesis [74, 75].

**Conclusions**

Somatic lesions in MPNs affect a diverse range of cellular functions, from cytokine signaling to histone modifications. To date, the \textit{JAK2} and \textit{MPL} mutations are the only genes that appear to show a high specificity for MPN and are also capable of inducing a myeloproliferative phenotype in animals. Many of the other MPN-associated genes are found in a wide range of myeloid malignancies and therefore are not considered to be specific for MPN. Further studies involving mouse models possessing one or combinations of these mutations may provide a deeper understanding into the development of MPN and how these mutations target the hematopoietic stem cells. In relation to the clonal evolution of the disease, it is clear that MPN is an extremely complex disease in regards to both its genotype and phenotype. Tailored and individualized diagnostic methods and therapies may be the only way forward in the future to treat this extremely complex disease successfully.

**Conflict of interest statement**

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**References**


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Robert Kralovics obtained his first degree in Molecular Biology and Genetics at the Comenius University in Bratislava and later his PhD in Biophysics at the Academy of Sciences of the Czech Republic in Brno. His research interests are myeloproliferative disorders and myeloid malignancies in general. One of his major achievements so far has been the identification of a gain-of-function mutation in the JAK2 kinase gene (V617F), which plays an important role in myeloproliferative disorders. He is an independent principal investigator at the Center for Molecular Medicine at the Austrian Academy of Sciences in Vienna.