Contemporary Management of

Myeloproliferative Neoplasms

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CHAPTER 2

Insights into Disease Pathogenesis of the Myeloproliferative Neoplasms

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INTRODUCTION

The myeloproliferative neoplasms (MPNs) are a heterogeneous group of malignant hematopoietic disorders which include polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and chronic myeloid leukemia (CML). The classification also includes other rare hematopoietic disorders, such as chronic neutrophilic leukemia, chronic eosinophilic leukemia, mastocytosis, and unclassified MPNs. All of these diseases are clonal hematopoietic disorders that result from the transformation of a pluripotent hematopoietic progenitor cell, which in turn causes increased hematopoiesis and an overproduction of normal and differentiated blood cells. In several subtypes, myeloproliferation occurs as a result of genetic changes that lead to cytokine independence. The type of blood cell that is preferentially amplified is specific to the disease: the granulocytic lineage (neutrophils, eosinophils, and basophils) in CML, the erythroid lineage in PV, the megakaryocytic lineage (and its derivative, platelets) in ET, and the granulocytic/megakaryocytic lineages in PMF.

In more than 95% of cases, CML is associated with the chromosomal translocation (9;22)(q34;q11.2), commonly known as the Philadelphia chromosome. The translocation leads to the presence of the oncogenic breakpoint cluster region (BCR)-Abelson leukemia virus (ABL) fusion protein in hematopoietic stem cells (HSCs). As a consequence of this fusion, there is constitutive activation of the ABL kinase and related pathways. The myeloproliferative chronic phase of the disease is followed by an accelerated phase during which normal maturation of hematopoietic cells ceases and transformation to acute myeloid leukemia (AML) occurs. This translocation is not seen in other forms of MPNs: thus, PV, ET, and PMF are commonly known as BCR-ABL negative MPNs.

In contrast to CML, whose genetic basis was described in 1973, the molecular pathogenesis of BCR-ABL negative MPNs remained poorly understood until the discovery of Janus kinase 2 (JAK2) V617F (a change of valine to phenylalanine at the 617 position) mutation in 2005.[1–5] The JAK2V617F alteration is present in PV, ET and PMF at different frequencies. Since this seminal discovery, a plethora of mutations have been identified in MPNs. Early investigations focused on mutations

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in signaling pathways and led to the identification of defects in myeloproliferative leukemia (*MPL*), *LNK*, casitas B-cell lymphoma (*CBL*), suppressor of cytokine signaling (SOCS) 1, SOCS2, and SOCS3, *NRAS*, and neurofibromatosis 1 gene (*NF1*) (Figure 2-1). Other genes implicated in pathogenesis of MPNs include transcription factors, such as *RUNX1* and *IKZF1* (IKAROS family zinc finger 1) and cell cycle and apoptosis regulators, including *RB1* and *TP53*. More recently, insights into the importance of epigenetic gene regulation have resulted in the discovery of new class of mutations. This latter group includes genes that are responsible for deoxyribonucleic acid (DNA) methylation and histone modifications such as ten-eleven-translocation 2 (*TET2*), additional sex combs-like protein 1 (*ASXL1*), enhancer of zeste homolog 2 (*EZH2*), isocitrate dehydrogenases 1 and 2 (*IDH1*, *IDH2*), and DNA (cytosine-5)-methyltransferase 3 alpha (*DNMT3A*) (Figure 2-2).

This chapter will focus on the molecular events implicated in the initiation, development and transformation of BCR-ABL negative disorders.

MUTATIONS IN GENES REGULATING SIGNAL TRANSDUCTION PATHWAYS

Janus Kinase 2

The JAK2 V617F mutation was identified in 2005 and currently represents the most prevalent mutation in BCR-ABL negative MPN. This mutation is present in 95–99% of PV, 50–70% of ET and 40–50% of PMF patients.[6] The JAK2 V617F mutation affects the non-catalytic pseudo-kinase domain (JH2) and is the result of a somatic G to C mutation, which leads to the substitution of a valine to phenylalaline at the 617 codon. The pseudo-kinase domain is required for inhibiting the basal activity of the JH1 kinase domain of JAK2. When the mutation is present, the protein remains phosphorylated even in the absence of any cytokine, which leads to the constitutive activation of downstream signaling pathways.

Janus kinase 2 interacts with three homodimeric receptors: (1) the erythropoietin receptor (EPO-R); (2) myeloproliferative leukemia (MPL) [the thrombopoietin (TPO) receptor]; and (3) granulocyte colony-stimulating factor (G-CSF) receptor. In normal cells, binding of cytokines to their respective receptors induces JAK2 phosphorylation, which results in recruitment of STAT (signal transducer and activator of transcription) proteins and activation of phosphoinositide-3-kinase (PI3K)-Akt [akt kinase (protein kinase B)] and the mitogen-activated protein kinase (MAPK) signaling pathways. Activation of this signaling cascade drives cell proliferation and survival. In the presence of JAK2 V617F, cells continue to proliferate and escape apoptosis.

Different mouse models have been developed to mimic the human diseases. In the first- generation model, bone marrow cells were retrovirally transduced by JAK2

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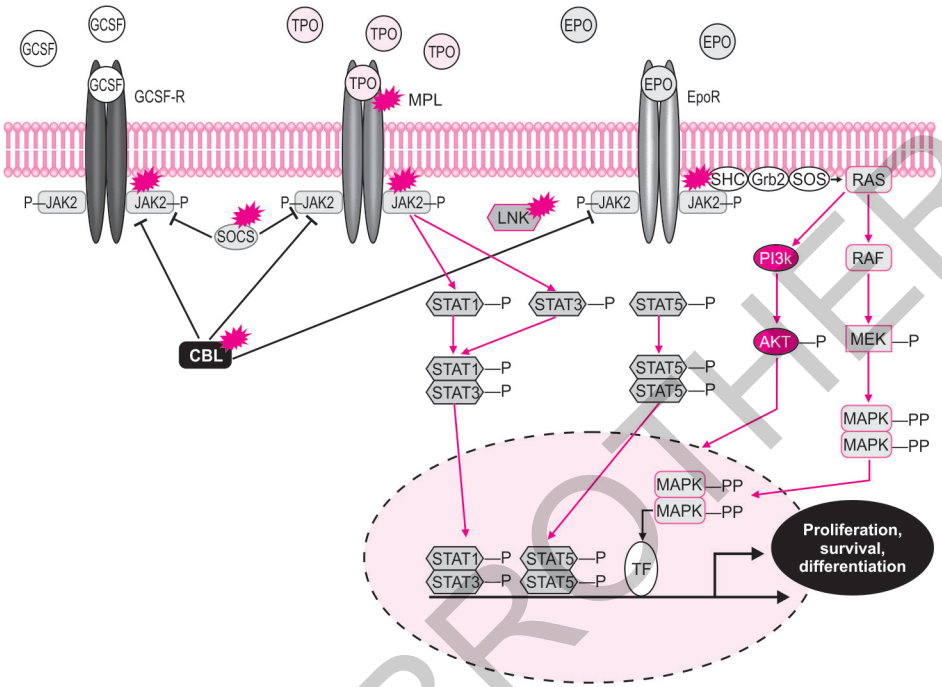

JAK, Janus kinase 2; STAT, signal transducer and activator of transcription; G-CSF, granulocyte colony-stimulating factor; TPO, thrombopoietin; EPO, erythropoietin; PI3K, phosphoinositide-3-kinase; Akt, akt kinase (protein kinase B); MAPK, mitogen-activated protein kinase; CBL, casitas B-cell lymphoma; SOCS, suppressor of cytokine signaling; MPL, myeloproliferative leukemia; MPNs, myeloproliferative neoplasms.

Figure 2-1 Schematic of JAK/STAT cytokine signaling pathways. Signaling downstream of G-CSF, TPO and EPO proceeds through activation of JAK2, which in turn activates STAT family members by phosphorylation. STAT dimers translocate to the nucleus to modulate gene expression, leading to survival, proliferation and differentiation. Binding of these cytokines to their cognate receptors also activates the PI3K/Akt and MAPK pathways. Signaling is negatively regulated by several factors including CBL, SOCS and LNK. Activating mutations in MPL and JAK2, as well as inactivating mutations in CBL, SOCS, and LNK are associated with the MPNs.

V617F and then transplanted to reconstitute lethally irradiated mice. In C57Bl6 mice, these cells principally lead to erythropoiesis and sometimes myelofibrosis (MF) with a long latency, while in BALB/c mice, the phenotype observed was more severe and was characterized by expanded erythropoiesis followed by myelofibrosis.[7,8] Multiple second-generation mouse models with knock-in mutations of JAK2 V617F showed a similar PV phenotype,[9-11] with the exception of one model whose disease more closely resembled ET.[12]

Although most PV patients carry the JAK2 V617F mutation, a minority are JAK2 V617F negative. Moreover, a larger proportion of PMF and ET patients are negative for this mutation, suggesting existence of other mutations in the JAK/STAT pathway.

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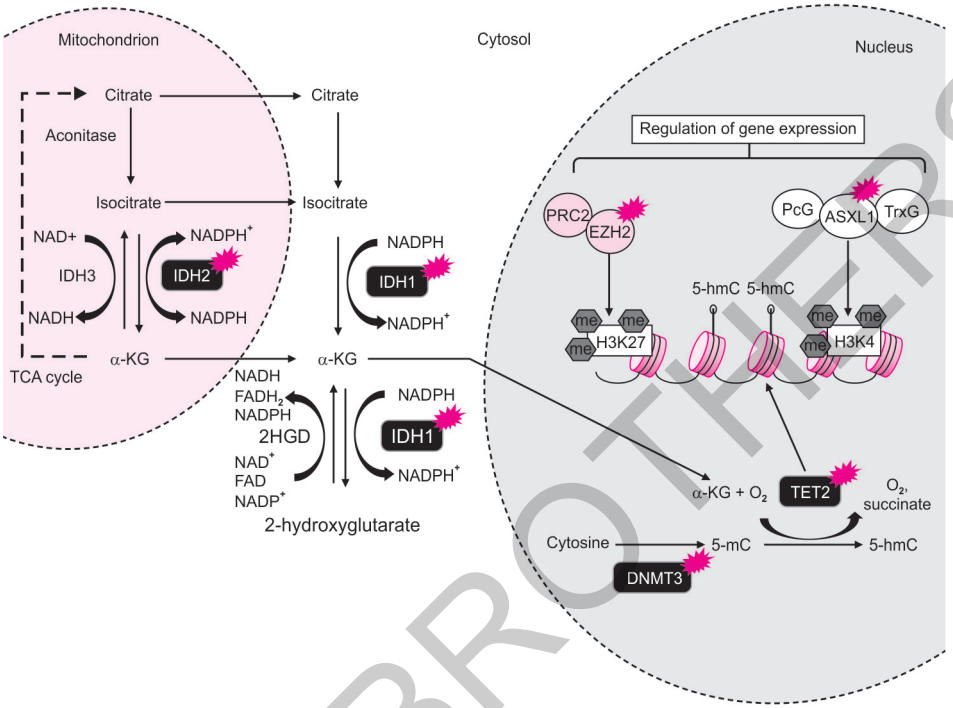

MPNs, myeloproliferative neoplasms; ASXL1, additional sex combs-like protein 1; EZH2, enhancer of zeste homolog 2; DNMT3A, DNA (cytosine-5)-methyltransferase 3 alpha; TET2, ten-eleven-translocation 2; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; PRC2, polycomb repressive complex 2; PcG, polycomb-group; 5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine; TCA, tricarboxylic acid.

Figure 2-2 Schematic of epigenetic regulators mutated in the MPNs. Genes that regulate gene expression by modification of chromatin that are also mutated in the MPNs include ASXL1, EZH2, DNMT3A and TET2. Other epigenetic factors that can be affected in the MPNs include the isocitrate dehydrogenases 1 and 2 (IDH1, IDH2). These enzymes catalyze the interconversion of isocitrate to α-ketoglutarate (αKG) and α-ketoglutarate (αKG) to 2-hydroxyglutarate (2HG), while simultaneously reducing nicotinamide adenine dinucleotide phosphate (NADP+) to NADPH. It is thought that the production of 2HG negatively regulates the activity of epigenetic regulators such as TET2.

Sequencing of JAK2 in JAK2 V617F negative patients identified other mutations in exon 12.[13,14] As observed with JAK2 V617F, these mutations result in EPO–independent colony growth and interleukin (IL)3-independent proliferation of Baf3 cells carrying these exon 12 mutations.

Because of the importance of JAK2 signaling in cell proliferation and survival, researchers searched for mutations in JAK2 associated receptors, leading to the identification of the activating MPLW515L [a somatic activating mutation in the transmembrane domain of MPL (W515L)] mutation. Interestingly, no mutations in

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EPO or G-CSF receptors were found in MPN patients. Other important mutations that have been discovered in MPN patients affect negative regulators of the signaling pathways, such as *CBL* and *LNK*.

Myeloproliferative Leukemia

Soon after the identification of JAK2 V617F, mutations in myeloproliferative leukemia (*MPL)* were found in approximately 5% of ET and 10% of PMF patients, but not in PV patients.[15,16] Of note, *MPL* mutations have also been reported in acute megakaryocytic leukemia and familial thrombocytosis.[17]

Myeloproliferative leukemia encodes the receptor for thrombopoietin, the cytokine that plays a role key in megakaryocyte survival, proliferation, and differentiation. The most frequent mutation affecting MPL is the MPLW515L substitution. Other substitutions in this codon have been described (MPLW515K and MPLW515A) but they represent a lower percentage.[18,19] Amino acid 515 is located in the intracellular region of MPL that lies immediately after the transmembrane domain. This region plays a major role in the cytosolic conformation of the receptor and prevents its autoactivation. Thus, mutations in this domain are sufficient to elicit spontaneous activation of the receptor and as a consequence, downstream signaling pathways.

As was previously observed in the case of JAK2 V617F, *MPL* mutations transform hematopoietic cells to factor-independent growth and induce JAK-STAT pathway activation.[16] However, even though *MPL* and *JAK2* mutations activate the same pathways and result in similar phenotypes *in vitro*, the *in vivo* models show striking differences. Indeed, expression of MPL W515L in mice leads to a lethal myeloproliferative disorder with thrombocytosis and MF.[16] This phenotype, therefore, mimics many of the phenotypes of human ET and PMF.

Casitas B-cell Lymphoma

The CBL protein family includes three homologs (c-CBL, CBL-b, and CBL-c) with ubiquitin ligase activity that targets different tyrosine kinases. The first mutation in *CBL* was described in AML patients.[20,21] Later, *CBL* mutations were identified in chronic myeloid disorders: 17% of chronic myelomonocytic leukemia (CMML) and less than 10% PMF are affected.[22,23] The main function of c-CBL is to participate in the ubiquitination of growth factor receptors, which induces their internalization and degradation.[24] Important targets of CBL are the fetal liver tyrosine kinase-3 receptor (*Flt3-R*), c-kit and MPL.

Mutations found in *CBL* affect two domains of the protein: the “linker” and the “ring”. The linker domain is necessary for interaction with the tyrosine kinase binding, while the ring domain, is essential for the negative regulatory function of CBL.[21] Then, the consequence of *CBL* mutations is loss of its inhibitory function.

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In vitro over-expression of the CBL mutant results in constitutive activation of the *Flt3* pathway and the phenotype of the cells resembles that of *Flt-3* mutated receptor tyrosine kinase.[25]

LNK

LNK, also known as Src homology (SH2) B3, belongs to a family of adaptor proteins. This family is characterized by the presence of three main domains: (1) proline-rich motifs in N-terminus; (2) a pleckstrin homology domain; and (3) an SH2 domain responsible for the negative activity of the protein. LNK is an intracellular protein, which acts as negative regulator of TPO and EPO pathways. Indeed, LNK negatively inhibits JAK2 through binding its SH2 domain.[26,27]

The murine LNK loss of function model displays an increased HSC pool with enhanced self-renewal capacity and increased quiescence,[28] two characteristics of HSCs controlled by TPO. In addition, $Lnk^{-/-}$ mice present abnormal megakaryopoiesis and erythropoiesis due to the absence of negative regulation of JAK/STAT signaling. This loss of control results in the development of thrombocytosis, splenomegaly and fibrosis.[29] In the MPNs, mutations in *LNK* are found in less than 5% of PMF and ET patients, but they are more frequently seen in patients that have undergone leukemic transformation.[30] Mutations almost always target a hot spot (codons 208 to –234) in the PH domain, which is believed to recognize phosphoinositide and control cell membrane localization.[31,32]

Suppressor of Cytokine Signaling

Another family of JAK2 negative regulators is Suppressor of Cytokine Signaling (SOCS). This group includes three members, *SOCS1*, *SOCS2,* and *SOCS3*.[33] These proteins bind JAK2 and inhibit its catalytic activity.[34] SOCS activity is regulated by methylation of their promoter. Hypermethylation of SOCS1 and SOCS3 promoters have been reported in JAK2 V617F PV and ET and in JAK2 V617F-negative and MPLW515F-negative ET.[35,36] In this condition, SOCS expression level is decreased.

Mutations in *SOCS1*, first described in B-cell lymphoma,[37] lead to their loss of function and, as a consequence, excess JAK/STAT signaling. In the MPNs, *SOCS1* is mutated in 14–25% of ET, 11–13% of PV, and 17% of PMF[35, 36] while *SOCS3* mutations are found in 10% of ET and 22% of PV[18,35,38,39] and *SOCS2* is mutated in 28% of MPNs.[40]

IMPLICATIONS

Currently, tests for *JAK2* and *MPL* mutations, but not other signal transduction genes, are available for clinical use. It should be noted that even though *JAK2* and *MPL* mutations are not found in all cases, JAK inhibitors are equally effective in mutation-positive and mutation-negative patients. This observation can be explained by the

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finding that patients without JAK2 or MPL mutations frequently show activated JAK/STAT signaling. As discussed above, this pathway can be activated by mutations in other genes, such as LNK. It is likely that screening for mutations in these other regulators will be made available for clinical use.

MUTATIONS IN EPIGENETIC REGULATORS

Epigenetic modifications include DNA methylation and post-translational modification of histone proteins. DNA methylation is involved in key cellular processes including X-chromosome inactivation, imprinting, and transcriptional silencing of specific genes and repetitive elements. DNA methylation patterns are frequently perturbed in human diseases, such as cancer and imprinting disorders including Beckwith–Wiedemann syndrome, Silver–Russell syndrome, Angelman syndrome, and Prader–Willi syndrome. Modifications of histones influence chromatin conformation, which regulates gene expression. As a consequence of these alterations, a wide range of cell metabolism and growth processes are impacted.

Ten-eleven-translocation 2

Ten-eleven-translocation 1, TET2, and TET3 are three homologous proteins, which convert 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC).[41,42] All three have two conserved C-terminal regions responsible for the catalytic activity. Contrary to TET1, TET2 and TET3 do not harbor a DNA- binding domain, suggesting that they interact with partner proteins to associate with DNA.

Ten-eleven-translocation TET1 was identified as the mixed lineage leukemia (MLL) partner AML-associated translocation t(10;11)(q22;q23), and named ten-eleven-translocation.[43] Later, the *TET2* location on chromosome (4q24) was described as a region of rearrangement in few cases of AML.[44] Mutations in *TET2* have been found thanks to whole genome array analysis and molecular cytogenetics. Mutations are present all along the coding sequence of the gene and they can be nonsense, missense and frameshift mutations.[45,46] In MPNs, approximately 16% of PV, 5% of ET and 17% of PMF patients carry a mutation in *TET2*. Mutations are also found in a wide range of myeloid malignancies, including 12% of myelodysplastic syndromes (MDS), 12% of myeloproliferative disorders and 22% of secondary AML.[45]

The main known function of TET2 is to convert 5-mC to 5-hmC. The role of 5-mC in gene regulation has been widely studied and it is associated with multiple effects on gene regulation, genomic imprinting, X-chromosome inactivation, and cancer development. The role of 5-hmC is less well understood, but several studies report that 5-hmC prevents the interaction of 5-mC with protein partners and represents a step toward cytosine demethylation.[47]

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To determine the role of TET2 in hematologic malignancies, short-hairpin ribonucleic acid (shRNAs) against the gene were introduced to into murine stem cells harvested from bone marrow. A reduction of TET2 level led to an increase in the proportion of hematopoietic stem/progenitor cells and a reduction of normal differentiation of granulocytes and monocytes.[48] Soon after, hematopoietic conditional knockouts of *TET2* were developed. These animals show an increase in stem cell self-renewal that is eventually accompanied by myeloproliferation with splenomegaly, monocytosis, and extramedullary hematopoiesis. Heterozygous mice also display increased stem cell self-renewal and extramedullary hematopoiesis.[49] A second knockout model of *TET2* has the same phenotype in Hematopoietic Stem Cells and a fatal disorder that mimics the human myeloid disorder. In this latter model, the authors also discovered defects in the lymphoid compartment with impairment in B and T cell differentiation.[50]

A recent study also reports that mutations in *TET2* can be detected in elderly individuals with clonal hematopoiesis, but without a detectable hematological malignancy. As observed in malignant context, in these healthy individuals the alterations in *TET2* are associated with modification of DNA methylation.[51] This study supports the model that *TET2* mutations enhance self-renewal and clonal expansion of HSCs when mutated. Furthermore, the data place mutations in *TET2* as potential early events in the development of hematologic malignancies. These results differ from those that describe *TET2* mutation as a late event in the progression of MPN to leukemic transformation[52-55] and suggest that a conserved order of genetic alterations is not required for MPN development.

Isocitrate Dehydrogenases 1 and 2

Isocitrate dehydrogenases 1 and 2 (IDH1, IDH2) are nicotinamide adenine dinucleotide phosphate ($NADP^+$) dependent enzymes, which catalyze the interconversion of isocitrate to α-ketoglutarate (αKG) and α-ketoglutarate (αKG) to 2-hydroxyglutarate (2HG), while simultaneously reducing nicotinamide adenine dinucleotide phosphate ($NADP^+$) to NADPH.[56,57] This reaction is required for protecting cells from oxidative stress-induced tumorigenesis. Mutations in *IDH1* and *IDH2* were first described in glioma[58] where they are mutually exclusive. Subsequently, they were found in 5–9% of AML and post-MPN AML. More recently, a comprehensive screen of 200 MPNs patients showed that nearly 21% of patients in blast-phase MPN carry a mutation in *IDH* while only 4% of PMF have an *IDH* mutation.[59] Furthermore, no mutations were found in PV and ET. Using a larger cohort of patients, however, another study reported *IDH* mutations in 0.8% of ET and 1.9% in PV.[60]

With respect to function, mutations in *IDH* affect arginine residues that are important for the activity of the protein (mutations R132S or R132G in IDH1 and

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codons R140 and R172 in IDH2). The presence of these mutated proteins leads to over-production of 2-hydroxyglutarate at the expense of α-ketoglutarate (αKG). As a consequence, αKG-dependent enzymes, including TET2, are affected, resulting in a decrease of 5-hmc among other changes.[48]

Additional Sex Combs-like Protein 1

Additional sex combs-like protein 1 (l *ASXL1* gene encodes the additional sex combs-like protein 1 and is located in chromosomal region 20q11. It belongs to a family of three enhancers of trithorax and polycomb proteins, which regulate the expression of developmental genes by chromatin remodeling.[61,62] ASXL1 has a double function since it is required to maintain both activation and silencing of Hox (homeobox) genes in flies and mice depending on the cell context.[62] The mechanism of action of ASXL1 remains poorly understood, but the protein is involved in chromatin organization through its participation in multiprotein complexes. One such complex is the polycomb repressive deubiquitinase complex, in which BAP1 (a ubiquitin carboxy-terminal hydrolase) removes monoubiquitin from histone 2A in nucleosomes.[63] In another complex, ASXL1 heterochromatin protein-1 (HP-1) associates with the histone demethylase LSD, which promotes repression of RAR (retinoic acid receptor)-mediated transcription.[62]

ASXL1$^{-/-}$ mice present a mild phenotype, although they do show defects in the frequency of differentiated lymphoid and myeloid progenitors. The absence of ASXL1 seems to have no effect on HSC, multipotent progenitors and peripheral blood. Notably, the mice do not appear to spontaneously develop myelodysplastic phenotypes or leukemia.[64]

Mutations in *ASXL1* include frameshift mutations, nonsense mutations, and large 20q11 deletions. These are found in 10–15% of MPNs (less than 7% of ET and PV, but 20–40% of PMF) and MDS, 40% of CMML and 15–20% of acute leukemia.[65,66] In most of the cases, mutations occur in exon 12 and result in synthesis of a truncated protein that lacks the PHD domain (C-terminal plant homeodomain), which is involved in protein-protein interactions.

Enhancer of Zeste Homolog 2

Enhancer of zeste homolog 2 is located on 7q36.1 and encodes for the PcG (polycomb-group) EZH 2 protein, which belongs to the polycomb repressive complex 2 (PRC2). This complex regulates cell proliferation, differentiation, cell-identity maintenance, aging, and plasticity by modification of chromatin structure.[67,68] Through the SET domain of EZH2, the PRC2 complex methylates histone H3 at lysine 27,[69] which is a marker of transcriptionally active status. EZH2 can also be associated with DNA-methyltransferases (DNMT) to contribute to DNA methylation regulation.

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Mutations in *EZH2* were first described in solid tumors, where they appear to induce dedifferentiation.[70] In myeloid disorders, missense, nonsense mutations, and premature codon stops are found all along the *EZH2* sequence. The mutations can be monoallelic or biallelic and, in contrast to solid tumors, they result in loss of function of the protein.[71,72] In the MPNs, *EZH2* mutations have been reported in 13% of patients with PMF, and 3% of PV. They are also described in MDS, demonstrating that *EZH2* mutations are not specific to a disease. In MDS, these mutations are usually associated with a poor prognosis.[73]

DNA (Cytosine-5)-Methyltransferase 3 Alpha

DNA (cytosine-5)-methyltransferase 3 alpha is a DNMT that catalyzes methylation of cytosines in genomic DNA. This methylation process plays an important role in gene repression and maintenance of active chromatin states, which in turn controls genomic imprinting, X-chromosome inactivation, and proper gene expression.[74,75] *DNMT3A* is located on chromosome 2p23.3 and is mutated in 4–22% of *de novo* AML[76] and approximately 8% of MDS patients.[77] A recent study showed that *DNMT3A* is mutated in 7% of PMF patients, but never found in CMML, blast-phase MPN's or post-PV/ET MF.[78] Nevertheless, larger cohort of patients is necessary to definitely rule out presence of *DNMT3A* mutation in these diseases. Functional studies to determine the effect of the mutations on DNMT3A function and hematopoiesis are underway.

Clinical Relevance

Currently, testing for mutations in these epigenetic regulators is not available for clinical use, but it is likely that future knowledge of the complete mutational profile will inform management of these patients. For example, recent studies have suggested that ASXL1 and IDH1/2 mutations may independently contribute to an adverse prognosis.[79,80] A more complete knowledge of the complete repertoire of mutations in patients may help identify those who would benefit from "epigenetic therapies" including hypomethylating agents and histone deacetylases (HDACs) (discussed in Chapter 8).

DISCUSSION

The discovery of JAK2 V617F mutation marked a huge advance in the understanding of the molecular biology of BCR-ABL negative MPNs, but it also presented new questions. The most important is how a single mutation can be responsible for the development of three different diseases. Even though many studies have provided novel insights, the question remains unanswered. Because the cell lineages impaired in PV, ET, and PMF are different from one another, it was first proposed that progenitor cells of the lineage uniquely harbored the mutation, which then led

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to the different phenotypes. However, evidence that ET, PV, and PMF are derived from stem cell clone able to give rise to granulocytic, erythroid, and megakaryocytic lineages rules out this model.[81]

Another model proposed that the mutation occurs in HSC, while the level and the duration of JAK2V617F expression determines the disease type.[82] In this model, low level of expression of the mutated kinase would lead an ET phenotype while high level of expression (consequence of a second event) would lead to PV. This model is supported by the examination of patient zygosity, which reveals that 93–100% of ET patients are heterozygous for JAK2 V617F, whereas 21–35% of PV and 0–21% of PMF are homozygous.[83] Animal models also support this dosage model.[84] Nevertheless, the presence of a *JAK2* mutation and its expression level alone are unable to explain the differential development of PV, PMF, and ET. Indeed, *JAK2* is not mutated in every patient. It is likely that the combination of additional mutations contributes to the specific phenotype.

A recent model of MPN development suggests that an initial inherited or acquired mutation, such as *TET2*, is present in the HSC leading to a premalignant state.[51] *ASXL1*, *TET2*, deletion of the chromosome 20q, which frequently precede *JAK2* mutation, may cause this pre-malignant condition. A second event, including mutagenesis of *JAK2*, *MPL*, *KIT,* or *CBL* may promote the MPN phenotype. More recently this model has been contested as several studies described *TET2* mutation as a late event in the progression of MPN to leukemic transformation.[52-55] Indeed, as in CML, BCR-ABL negative MPNs have the propensity to evolve to AML. Thus, MPN can be considered as a transition state that, with the addition of other events, leads to leukemic transformation.

Finally, a recent study argues that the order in mutational events can vary among patients.[85] Additionally, the effects of *TET2* or *ASXL1* mutations are different depending on when they occur, during the MPN pathogenesis or leukemic transformation.[54] Thus, a better understanding of the pathogenesis of MPN and molecular events involved in leukemic transformation are necessary to give a new direction in drug discovery research. Although ruxolitinib is approved for use as a therapy for Primary Myelofibrosis,[86] it is unable to cure the disease.[87] Thus, the best hope for patients lies in the development of rational combinations of other agents, such as immunotherapy (lenalidomide, thalidomide, and pomalidomide), interferons, and epigenetic therapies (azacitidine, decitabine, and HDAC inhibitors) with a JAK inhibitor. The best therapies could then be tailor-made to target the specific set of mutations in an individual patient.

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