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The Molecular Genetics of Myeloproliferative Neoplasms

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Activated JAK-STAT signaling is central to the pathogenesis of *BCR-ABL*-negative myeloproliferative neoplasms (MPNs) and occurs as a result of MPN phenotypic driver mutations in *JAK2*, *CALR*, or *MPL*. The spectrum of concomitant somatic mutations in other genes has now largely been defined in MPNs. With the integration of targeted next-generation sequencing (NGS) panels into clinical practice, the clinical significance of concomitant mutations in MPNs has become clearer. In this review, we describe the consequences of concomitant mutations in the most frequently mutated classes of genes in MPNs: (1) DNA methylation pathways, (2) chromatin modification, (3) RNA splicing, (4) signaling pathways, (5) transcription factors, and (6) DNA damage response/stress signaling. The increased use of molecular genetics for early risk stratification of patients brings the possibility of earlier intervention to prevent disease progression in MPNs. However, additional studies are required to decipher underlying molecular mechanisms and effectively target them.

Myeloproliferative neoplasms (MPNs) are a collection of hematopoietic disorders characterized by excessive proliferation of hematopoietic cells of the myeloid lineage. MPNs are classified based on the presence (chronic myeloid leukemia [CML]) or absence of the *BCR-ABL* translocation. In the majority of cases, *BCR-ABL*-negative MPN is caused by somatic mutations that arise in the hematopoietic stem cell (HSC) compartment and activate the JAK-STAT signaling pathway (Mead and Mullally 2017). *BCR-ABL*-negative MPN is the focus of this review and will be referred to as MPN for the remainder of the review. The JAK2-STAT signaling cascade is activated on binding of ligands to type I cytokine receptors including MPL (thrombopoietin [TPO] receptor), the erythropoietin (EPO) receptor, and the granulocyte colony-stimulating factor receptor (G-CSF) receptor. Mutations that activate the JAK-STAT signaling pathway render hematopoietic stem and progenitor cells (HSPCs) hypersensitive to cytokines. Different subtypes of MPN are classified descriptively based on the predominant lineages involved: elevated platelet count in essential thrombocythemia (ET); elevated red

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blood cell count in polycythemia vera (PV); megakaryocytic hyperplasia and bone marrow fibrosis in myelofibrosis (MF). ET and PV can evolve into MF (termed secondary MF) or patients may present with de novo MF (termed primary MF or PMF). All subtypes of MPN can transform into secondary acute myeloid leukemia (sAML), which is typically refractory to chemotherapy and generally carries a poor prognosis.

MUTATIONS IN THE JAK-STAT SIGNALING PATHWAY

Mutations that activate the JAK-STAT signaling pathway (Fig. 1) are disease-initiating in MPNs and consequently are termed MPN phenotypic driver mutations. The most frequent MPN phenotypic driver mutation occurs in *JAK2*, a gene encoding a nonreceptor tyrosine kinase. The mutation results in a valine to phenylalanine substitution at position 617 (V617F) in the JH2 domain of the protein, negating the otherwise repressive function of this domain on kinase activity (Chen and Mullally 2014). JAK2 acts as an intermediate signaling molecule for several growth factor receptors including myeloproliferative leukemia (MPL), the EPO receptor, and G-CSF receptor. As a consequence, patients with *JAK2* mutations may have thrombocytosis (ET, 50%–60% of patients have a $JAK2^{V617F}$ mutation; see Table 1), erythrocytosis (PV, 95% of patients have a $JAK2^{V617F}$ mutation), and leukocytosis (observed in PV). *JAK2* exon 12 mutations, occurring in 2%–3% of PV patients, also result in constitutive activation of JAK2 signaling and typically present with isolated erythrocytosis (Scott et al. 2007). The second most frequent MPN phenotypic driver mutation is found in the endoplasmic reticulum chaperone,

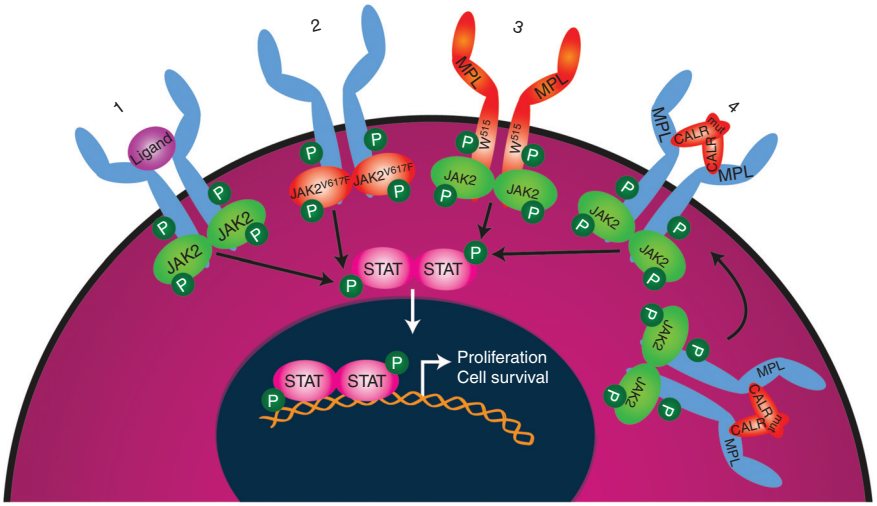

Figure 1. Mutations activating the JAK-STAT signaling pathway are sufficient to cause myeloproliferative neoplasm. In the normal situation (1), the JAK-STAT signaling pathway is activated on binding of a ligand to type I cytokine receptors including MPL (thrombopoietin [TPO] receptor), the erythropoietin (EPO) receptor, and granulocyte colony-stimulating factor (G-CSF) receptor. Activation of the JAK-STAT signaling pathway causes cell proliferation and survival of the relevant myeloid lineage cells. When JAK2 is constitutively active owing to mutations such as V617F (2), downstream signaling from type I cytokine receptors is increased, leading to clonal expansion of hematopoietic stem cells (HSCs), increased erythropoiesis (EPOR), thrombopoiesis (MPL), and granulopoiesis (G-CSFR). Situation (3) shows that MPL mutations at position W515 activate JAK-STAT signaling. In situation (4), mutant CALR develops a pathogenic binding interaction with MPL (but not with EPO or G-CSF receptor) that activates JAK-STAT signaling. In situations (3) and (4), because only the MPL receptor is involved, JAK-STAT pathway activation is restricted to MPL-expressing hematopoietic stem cell (HSC) and megakaryocyte lineage cells resulting in thrombocytosis. Mutated proteins are depicted in orange. P, Phosphorylation.

Table 1. Overview of the most frequent mutations found in MPNs and post-MPN AML

Gene	Most frequent mutation (types)	Mutation frequency				References
		ET	PV	(P)MF	Post-MPN AML	
MPN phenotypic driver mutations						
JAK2	Heterozygous and homozygous (acquired uniparental disomy) exon 12 and exon 14 mutations. V617F, located in the repressive JH2 domain, is the most frequent mutation. Also, gene/chromosomal duplications are found.	50%–60%	95%	50%–60%	37%–60%	Scott et al. 2007; Abdel-Wahab et al. 2010; Zhang et al. 2012; Vannucchi et al. 2013; Guglielmelli et al. 2014; Lundberg et al. 2014; Tefferi et al. 2016a,b; Lasho et al. 2018
CALR	Heterozygous insertion and/or deletions in exon 9, resulting in a +1 base pair frameshift and a novel carboxyl terminus.	26%	<1%	18%–32%	21%–25%	Guglielmelli et al. 2014; Lundberg et al. 2014; Rampal et al. 2014; Tefferi et al. 2016a,b: Lasho et al. 2018
MPL	Heterozygous missense mutations, W515L/K most frequent, resulting in constitutive activation of the receptor.	4%	<1%	6%–9%	8%–13%	Zhang et al. 2012; Vannucchi et al. 2013; Lundberg et al. 2014; Guglielmelli et al. 2014; Tefferi et al. 2016a,b; Lasho et al. 2018
DNA methylation						
TET2	Heterozygous or homozygous loss-of-function mutations in its catalytic domain, causing reduced conversion of methylated to hydroxymethylated cytosines.	7%–16%	19%–22%	10%–18%	19%–28%	Abdel-Wahab et al. 2010; Zhang et al. 2012; Vannucchi et al. 2013; Lundberg et al. 2014; Tefferi et al. 2016a,b; Lasho et al. 2018; Venton et al. 2018
IDH1[a]/ *IDH2*[a]	Heterozygous missense mutations in the active catalytic site, IDH1: R132, IDH2: R140 and R172, causing acquisition of the ability to convert αKG into 2-hydroxyglutatate.	1%	2%	0%–6%	19%–31%	Abdel-Wahab et al. 2010; Pardanani et al. 2010; Tefferi et al. 2010, 2016a,b; Zhang et al. 2012; Vannucchi et al. 2013; Guglielmelli et al. 2014; Lundberg et al. 2014; Rampal et al. 2014; Lasho et al. 2018; Venton et al. 2018
DNMT3A	Mutations in *DNMT3A* in MPN occur as nonsense/ frameshift mutations in addition to missense mutations (including at R882, which is located in the methyltransferase domain), resulting in reduced methyltransferase activity.	0%–9%	0%–7%	3%–15%	2%–14%	Abdel-Wahab et al. 2011; Lin et al. 2011; Stegelmann et al. 2011; Zhang et al. 2012; Vannucchi et al. 2013; Lundberg et al. 2014; Wang et al. 2014; Tefferi et al. 2016a,b: Lasho et al. 2018; Venton et al. 2018

Continued

Table 1. *Continued*

Gene	Most frequent mutation (types)	Mutation frequency				References
		ET	PV	(P)MF	Post-MPN AML	
Chromatin modification						
ASXL1[a]	Heterozygous nonsense and frameshift mutations in exon 12 leading to loss of its PHD domain.	1%–11%	3%–12%	18%–37%	17%–47%	Tefferi et al. 2016a,b, 2018d; Lasho et al. 2018; Vannucchi et al. 2013; Abdel-Wahab et al. 2010; Zhang et al. 2012; Lundberg et al. 2014; Guglielmelli et al. 2014; Rampal et al. 2014; Venton et al. 2018
EZH2[a]	Heterozygous/homozygous/hemizygous loss-of-function mutations that disrupt or delete the catalytic SET2 domain.	1%–3%	0%–3%	0%–9%	13%–15%	Vannucchi et al. 2013; Guglielmelli et al. 2014; Lundberg et al. 2014; Tefferi et al. 2016a,b; Lasho et al. 2018; Venton et al. 2018
RNA splicing						
SRSF2[a]	Heterozygous missense mutations and small in-frame deletions around hotspot P95, affecting the preferred RNA recognition sequence (wild-type GGNG and CCNG, mutant GGNG) in RNA exon splicing enhancers.	2%	3%	8%–18%	13%–22%	Zhang et al. 2012; Vannucchi et al. 2013; Guglielmelli et al. 2014; Rampal et al. 2014; Tefferi et al. 2016a,b, 2018d; Lasho et al. 2018; Venton et al. 2018
U2AF1[a]	Heterozygous missense mutations around hotspots S34 and Q157, which alter the preferred –3 (C/A >> T, S34F/Y) or +1 (G >> A, Q157P) nucleotides flanking the AG of the 3′ splice site.	1%	<1%	16%	5%–6%	Zhang et al. 2012; Tefferi et al. 2016a,b, 2018; Lasho et al. 2018d
ZRSR2	Hemizygous frameshift and nonsense mutations. Loss-of-function mutations.	3%	5%	10%	2%	Zhang et al. 2012; Tefferi et al. 2016a,b
SF3B1	Heterozygous missense mutations in exons 14–16, hotspot K700E most frequent mutation. Most commonly mutated in MDS/MPN-RS-T 80%.	5%	3%	9%–10%	4%–7%	Dunbar et al. 2008; Makishima et al. 2009; Muramatsu et al. 2010; Malcovati et al. 2011; Papaemmanuil et al. 2011; Yoshida et al. 2011; Zhang et al. 2012; Tefferi et al. 2016a,b, 2018d; Lasho et al. 2018
Signaling						
LNK	Mostly heterozygous missense substitutions targeting the pleckstrin homology domain in 50% of cases.	1%–3%	0%–9%	0%–6%	11%	Lundberg et al. 2014; Tefferi et al. 2016a,b; Lasho et al. 2018

Continued

Table 1. *Continued*

Gene	Most frequent mutation (types)	Mutation frequency				References
		ET	PV	(P)MF	Post-MPN AML	
CBL	Homozygous missense substitutions located in the RING and linker domain reducing E3 ligase activity.	0%–1%	0%–2%	0%–6%	4%	Grand et al. 2009; Vannucchi et al. 2013; Lundberg et al. 2014; Tefferi et al. 2016a,b; Lasho et al. 2018
NRAS/ KRAS	Heterozygous missense substitutions at codons 12, 13, and 61 causing reduced intrinsic GTP hydrolysis and resistance to GAPs.	<1%	0%–1%	3%–4%	7%–15%	Zhang et al. 2012; Lundberg et al. 2014; Tefferi et al. 2016a,b; Lasho et al. 2018; Venton et al. 2018
PTPN11	Heterozygous missense mutations in the Src-homology 2 (N-SH2) and phosphotyrosine phosphatase (PTP) domains causing increased phosphatase activity.	0%–2%	<1%	0%–2%	6%–8%	Ding et al. 2009; Lundberg et al. 2014; Rampal et al. 2014; Tefferi et al. 2016a,b; Lasho et al. 2018
Transcription factors						
RUNX1	Missense, frameshift, and nonsense mutations causing loss of function, and may act in a dominant-negative fashion over wild-type RUNX1. Both homozygous and heterozygous mutations are found.	0%–2%	0%–2%	3%–4%	4%–13%	Ding et al. 2009; Zhang et al. 2012; Lundberg et al. 2014; Tefferi et al. 2016a,b: Lasho et al. 2018; Venton et al. 2018
NFE2	Mostly heterozygous frameshift mutations causing increased expression and protein levels of wild-type NFE2.	<1%	2%–3%	0%–3%	<1%	Jutzi et al. 2013; Lundberg et al. 2014
DNA repair response/stress signaling						
TP53	Mostly missense mutations, both alleles affected. Possibly loss-of-function, gain-of-function, and/or dominant-negative.	2%–6%	1%	1%–3%	11%–36%	Harutyunyan et al. 2011; Zhang et al. 2012; Lundberg et al. 2014; Rampal et al. 2014; Tefferi et al. 2016a,b; Lasho et al. 2018; Venton et al. 2018
PPM1D	Heterozygous exon 6 mutations causing loss of carboxy-terminal degradation domain.	2%	1%	1%	NA	Grinfeld et al. 2018

MPN, Myeloproliferative neoplasm; AML, acute myeloid leukemia; ET, essential thrombocythemia; PV, polycythemis vera; (P)MF, (primary) myelofibrosis; ASXL1, EZH2, SRSF2, IDH1/2, U2AF^{Q157}, GTPase-activating proteins; αKG, α-ketgoglutarate; MDS/MPN-RS-T, myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; NA, not assessed; PHD, plant homeodomain.

[a]High molecular risk mutations in (primary) myelofibrosis (Vannucchi et al. 2013; Guglielmelli et al. 2018).

CALR. *CALR* mutations occur as heterozygous insertion and/or deletions in exon 9, resulting in a +1 base pair frameshift (Klampfl et al. 2013; Nangalia et al. 2013). This frameshift changes the reading frame and results in the generation of a novel mutant-specific carboxyl terminus. CALR induces MPN by binding to and activating MPL signaling in a TPO-independent manner (Elf et al. 2016). Consistent with the observation that MPL is mostly expressed in HSCs and the megakaryocytic lineage, mutations in *CALR* occur in ET (26%) and MF but not typically in PV. The third most frequent MPN phenotypic driver mutation occurs in *MPL*, with the majority of mutations located at tryptophan 515 (W515L/K) (Pikman et al. 2006). *MPL* mutations primarily affect the megakaryocytic lineage and are present in ET (4%) and MF, but not in PV. Mutational frequencies in PMF are 50%–60% for *JAK2* mutations, 18%–32% for *CALR*, and 6%–9% for *MPL* (Table 1). Additionally, ~10% of ET and PMF patients are "triple negative"—they do not harbor *JAK2*, *CALR,* nor *MPL* mutations (Angona et al. 2016; Milosevic Feenstra et al. 2016).

It is important to note that mutations in *JAK2*, *CALR*, or *MPL* are sufficient "alone" to engender MPNs in people and in mice (Pikman et al. 2006; Mullally et al. 2013; Li et al. 2018). In 45%–50% of MPN cases, an MPN phenotypic driver mutation is the sole mutation identified based on our current knowledge of genes known to be somatically mutated in myeloid malignances (Lundberg et al. 2014; Grinfeld et al. 2018), and in a recent study all MPN patients of <39 years old had *JAK2*, *CALR*, or *MPL* as the sole mutation identified (Grinfeld et al. 2018). Furthermore, *JAK2*^{V617F} is a common mutational driver of clonal hematopoiesis of indeterminate potential (CHIP), an entity in which clonally restricted somatic mutations in genes associated with hematological malignancies are found in normal individuals (Gibson and Steensma 2018). In virtually all cases of JAK2^{V617F} CHIP, the *JAK2* mutation occurs as an isolated event indicating that JAK2^{V617F} *alone* is sufficient to cause clonal hematopoiesis (Jaiswal et al. 2014). The prevalence of JAK2^{V617F} MPN is significantly lower than that of JAK2^{V617F} CHIP (Hinds et al. 2016), suggesting there are factors that constrain and promote the transition from JAK2^{V617F} clonal hematopoiesis to MPN. Recent studies indicate that JAK2^{V617F} CHIP is itself a clinically relevant entity, being associated with an increased risk of both atherosclerotic heart disease (Jaiswal et al. 2017) and venous thrombosis (Wolach et al. 2018). These data suggest that JAK2 mutations can cause a continuum of clinically relevant disease entities, from premalignant clonal expansion to all three classical MPNs.

OTHER SOMATIC GENE MUTATIONS

Besides the three JAK-STAT activating MPN phenotypic driver mutations, >50% of MPN patients harbor additional somatic mutations (Table 1) (Grinfeld et al. 2018). Figure 2 shows an illustrative example of the acquisition of somatic mutations in different hematopoietic states (e.g., polyclonal hematopoiesis, CHIP, MPN, and post-MPN AML). The most common classes of concomitant somatic mutations found in MPN patients are (1) DNA methylation (*TET2*, *IDH1*, *IDH2*, *DNMT3A*), (2) chromatin modification (*ASXL1*, *EZH2*), (3) RNA splicing (*SRSF2*, *U2AF1*, *SF3B1*, and *ZRSR2*), (4) signaling pathways (*LNK*/*SH2B3*, *CBL*, *NRAS*, *KRAS*, *PTPN1*), (5) transcription factors (*RUNX1* and *NFE2*), and (6) DNA damage response/stress signaling (*TP53*, *PPM1D*) (Fig. 3). Below, we describe in which MPN subtypes these mutations are found and how specific mutations affect gene function and contribute to MPNs.

DNA METHYLATION

TET2

Consequences of Mutations on Gene Function

During DNA replication, methylated cytosines are either replaced passively by unmethylated cytosines or actively after a process of chemical modifications initiated by ten eleven translocation (TET) proteins. The TET2 enzyme catalyzes oxidation of 5-methylcytosine to 5-hy-

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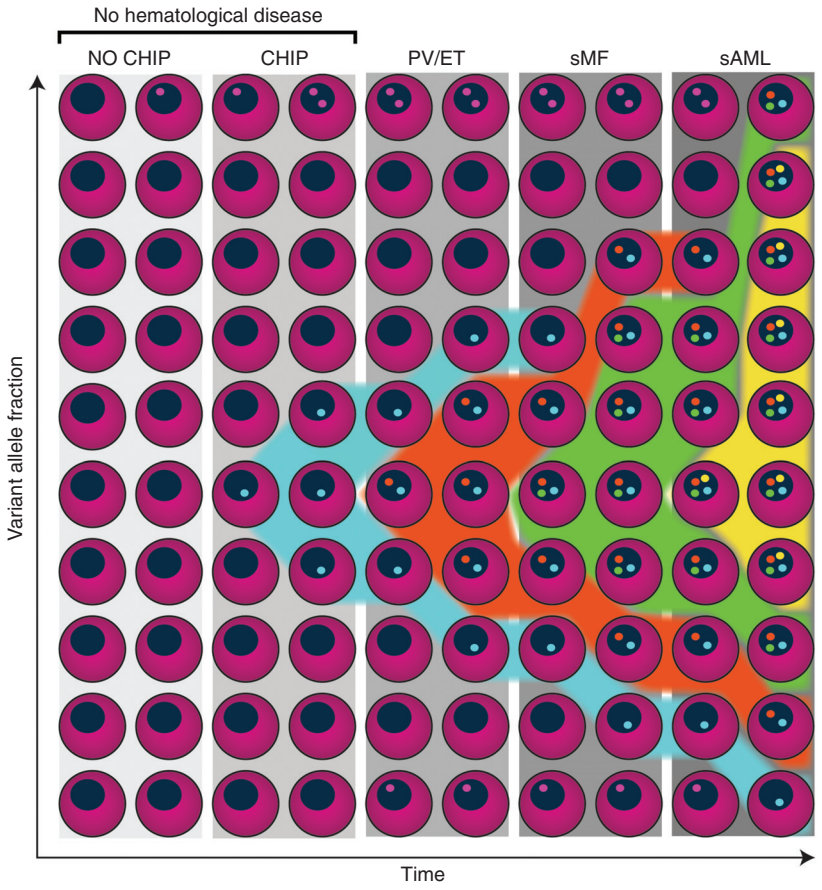

Figure 2. Illustrative example of an individual case with clonal hematopoiesis and myeloproliferative neoplasm (MPN) disease progression. Individuals without hematological diseases may have somatic mutations in genes that do not impact hematopoiesis (i.e., passenger mutations, indicated with pink dots). At some point, the individual case represented in this figure acquires a mutation (e.g., in *DNMT3A*) that causes clonal expansion of the hematopoietic stem cell (HSC) without necessarily causing an overt hematological disease (i.e., clonal hematopoiesis of indeterminate potential [CHIP]), indicated with blue dots and expansion of the blue clone from 10% to 30%. Subsequently, this individual acquires one of the MPN phenotypic "driver" mutations (e.g., $JAK2^{V617F}$, indicated with orange dots), that results in the development of an MPN (e.g., polycythemia vera [PV] or essential thrombocythemia [ET]). Chronic phase MPN (i.e., PV or ET) may then progress to secondary myelofibrosis (sMF) on acquisition of additional mutations (e.g., a mutation in EZH2 [indicated with green dots]). This clone expands over time and, finally, overt secondary acute myeloid leukemia (sAML) may arise on acquisition of a TP53 mutation (yellow dots).

droxymethylcytosine (5-hmc). TET proteins depend on α-ketoglutarate (αKG), Fe^{2+}, and ascorbate for their dioxygenase activity (Tahiliani et al. 2009; Minor et al. 2013). Mutations in *TET2* are loss-of-function causing impaired catalytic function, resulting in reduced levels of 5-hmc and resultant DNA hypermethylation (Fig. 3).

Clinical Data

All types of mutations and deletions are found throughout the *TET2* gene, with the majority of somatic missense mutations found in its catalytic domain. *TET2* mutations occur in 7%–22% of MPNs and 19%–28% of post-MPN AML patients (Table 1). Although some studies

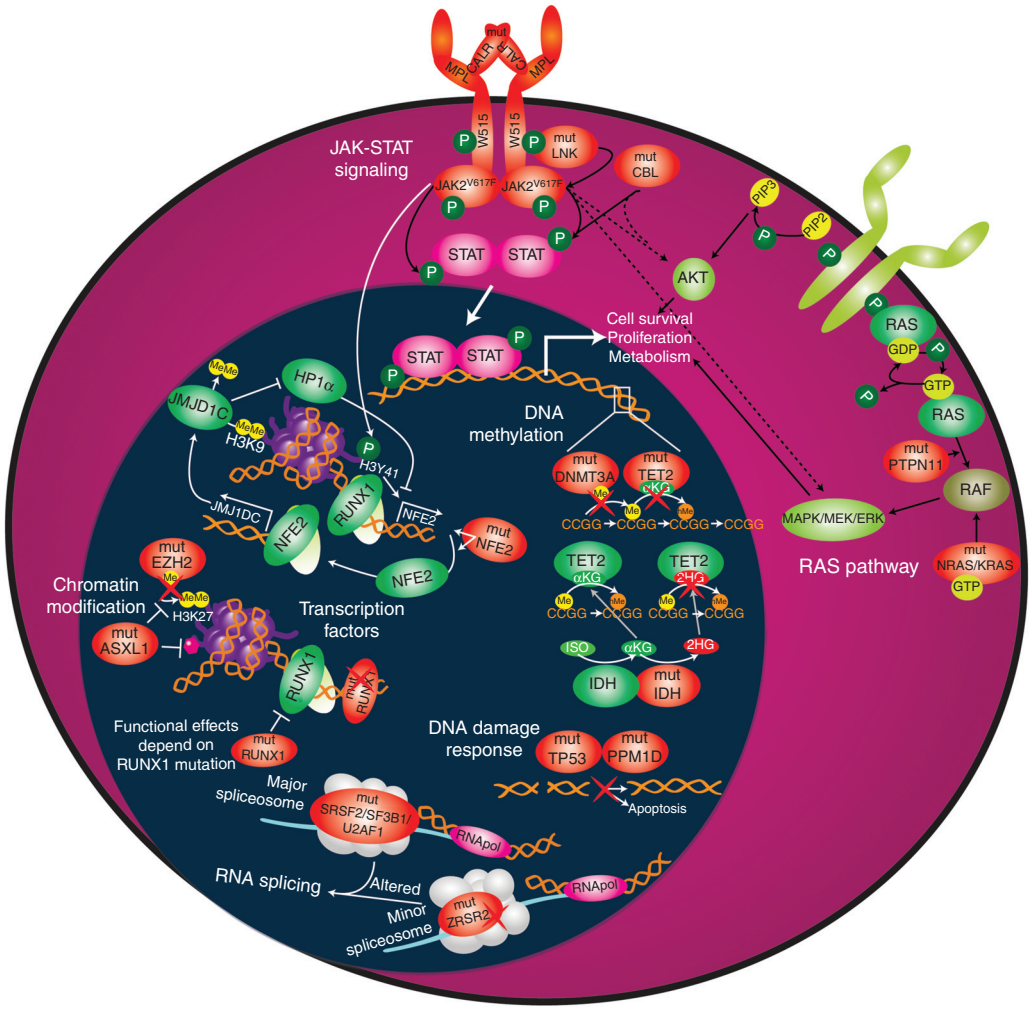

Figure 3. Overview of classes of genes found mutated in myeloproliferative neoplasms (MPNs). This includes (1) DNA methylation (*TET2*, *IDH1*, *IDH2*, and *DNMT3A*), (2) chromatin modification (*ASXL1* and *EZH2*), (3) RNA splicing (*SRSF2*, *U2AF1*, *SF3B1*, and *ZRSR2*), (4) JAK-STAT and RAS signaling pathways (*JAK2*, *CALR*, *MPL*, *LNK*/*SH2B3*, *CBL*, *NRAS*, *KRAS*, and *PTPN1*), (5) transcription factors (*RUNX1* and *NFE2*), and (6) DNA damage response/stress signaling (*TP53* and *PPM1D*). Wild-type proteins are depicted in green and mutated (mut) proteins are depicted in orange. For illustration, all genes/proteins described in this review are depicted, although some mutations may actually lead to loss of protein expression. (Refer to the main text for detailed explanations of the depicted processes.) αKG, α-ketoglutarate; GDP, guanosine diphosphate; GTP, guanosine triphosphate; 2HG, 2-hydroxyglutarate; hMe, hydroxymethyl; ISO, isocitrate; Me, methyl; P, phosphorylation; PIP2, phosphatidylinositol(4,5)-biphospate; PIP3, phosphatidylinositol(3,4,5)-triphosphate; RNApol, RNA polymerase.

have suggested that *TET2* mutations increase the risk of leukemic transformation (Abdel-Wahab et al. 2010; Beer et al. 2010; Zhang et al. 2012; Lundberg et al. 2014), more recent studies have not consistently seen this association (Tefferi et al. 2016b; Lasho et al. 2018). *TET2* mutations are the most common co-occurring mutation with the JAK2^{V617F} mutation. The order of acquisition of *JAK2* and *TET2* mutations impacts the MPN phenotype, with JAK2-first patients having a higher probability of presenting with PV than ET and a

higher risk of thrombosis (Ortmann et al. 2015).

Mouse Models and Affected Cellular Pathways

In murine and xenograft HSC repopulation studies, *Tet2* knockout/knockdown/mutated HSCs have superior HSC repopulation as compared with *Tet2* wild-type cells and JAK2^{V617F+}/TET2 knockout/knockdown/mutated cells have superior HSC repopulation as compared with JAK2^{V617F+}-only HSCs (Delhommeau et al. 2009; Chen et al. 2015; Kameda et al. 2015b). When comparing JAK2^{V617F}-induced MPN with JAK2^{V617+}*Tet2* knockout/knockdown MPN, the later mice developed a more severe form of MPN and had reduced survival (Chen et al. 2015; Kameda et al. 2015b). Proposed mechanisms by which *TET2* mutations lead to more severe forms of MPN include epigenetic repression of tumor suppressor genes and increased expression of HSC self-renewal genes possibly due to enhancer hypermethylation (Kameda et al. 2015a; Rasmussen et al. 2015).

IDH1/IDH2

Consequences of Mutations on Gene Function

Wild-type isocitrate dehydrogenase ($NADP^+$) 1 and 2 (IDH1/IDH2) are metabolic enzymes that catalyze the oxidative decarboxylation of isocitrate to αKG, the cosubstrate for TET proteins. Because of missense mutations in the active catalytic site of IDH (IDH1: R132, IDH2: R140 or R172), IDH proteins acquire the ability to further convert αKG into 2-hydroxyglutatate (Fig. 3). Besides αKG, 2-hydroxyglutatate can also bind TET proteins and the Jumonji family of H3K9 and H3K36 demethylases (Xu et al. 2011). However, because 2-hydroxyglutatate cannot function as a cosubstrate, it inhibits the function of these enzymes.

Clinical Data

Missense mutations in *IDH1* and *IDH2* occur at low frequencies in MPNs and in 19%–31% of post-MPN AML (Table 1). *IDH1/2* mutations have been associated with worse prognosis in ET, and in PMF with a high risk for subsequent transformation to post-MPN sAML (Tefferi et al. 2010, 2016b; Guglielmelli et al. 2014; Yonal-Hindilerden et al. 2016).

Mouse Models and Affected Cellular Pathways

Like *Tet2* loss, expression of mutant IDH1 causes an expansion of myeloid progenitors in mouse models (Sasaki et al. 2012). Both Jak2^{V617F}-only and Jak2^{V617F}/Idh2^{R140Q} mice develop a PV phenotype with splenomegaly, but Jak2^{V617F}/Idh2^{R140Q} mice show blast-like cells in the spleen and disruption of splenic architecture that is not observed in Jak2^{V617F}-only mice (McKenney et al. 2018a). Overt leukemia was not seen in Jak2^{V617F}/Idh2^{R140Q} mice, suggesting that additional factors are required to induce leukemic transformation in Jak2^{V617F}-expressing mice.

DNMT3A

Consequences of Mutations on Gene Function

DNA methyl transferase (DNMT)3A and DNMT3B are de novo methyltransferases as opposed to DNMT1, a maintenance methyltransferase that ensures that DNA methylation is inherited by the daughter cell after cell division. Mutations in *DNMT3A* in MPN occur as nonsense/frameshift mutations, in addition to missense mutations (including at R882), resulting in reduced methyltransferase activity (Fig. 3) (Ley et al. 2010; Yamashita et al. 2010; Abdel-Wahab et al. 2011; Emperle et al. 2018). It was suggested that DNMT3A^{R882mut} acts in a dominant-negative fashion by inhibiting the function and/or oligomerization of wild-type DNMT3A, but recent evidence contradicts this finding (Emperle et al. 2018).

Clinical Data

DNMT3A mutations are found in 7% of MPNs, with lower frequency in ET and PV compared

with (P)MF and post-MPN AML (Table 1). Patients are more likely to present with ET compared with PV or MF when *DNMT3A* mutations are acquired before $JAK2^{V617F}$ compared with patients who first acquired $JAK2^{V617F}$ (Nangalia et al. 2015).

Mouse Models and Affected Cellular Pathways

Human *DNMT3A/JAK2* and *DNMT3A/MPL* double-mutant subclones have a growth advantage compared with *JAK2* and *MPL* single-mutant subclones (Rao et al. 2012; Nangalia et al. 2015). This is consistent with in vitro serial replating assays and in vivo CRISPR knockout of *Dnmt3a* in $Jak2^{V617F}$-expressing mice (Jacquelin et al. 2018). When followed for 32 weeks, mice transplanted with $Jak2^{V617F}$ control HSPCs showed a PV phenotype, whereas recipients receiving $Jak2^{V617F}$ Cas9 Dnmt3a sgRNA HSPCs first developed a PV-like disease, but had progressed into MF by 32 weeks of age (Jacquelin et al. 2018). Mice transplanted with $Jak2^{V617F}$/Dnmt3a KO HSPCs showed a marked reduction in long-term and short-term HSCs and a relative accumulation of multipotent progenitors compared with $Jak2^{V617F}$ control recipients (Jacquelin et al. 2018). Mechanistic studies in this model indicate that *DNMT3A* mutations contribute to MPN progression by deregulating Polycomb repressive complex 2 (PRC2)-mediated gene expression, through enhanced chromatin accessibility at active enhancer sites (Jacquelin et al. 2018).

CHROMATIN MODIFICATION

ASXL1

Consequences of Mutations on Gene Function

Additional sex combs like 1, transcriptional regulator (ASXL1) interacts with several chromatin-modifying proteins, including PRC2. Mutations in *ASXL1* are almost exclusively heterozygous nonsense and frameshift mutations in exon 12. There is some controversy regarding the consequences of these mutations on ASXL1 function, which could be loss- or gain-of-function and/or a dominant-negative effect. *ASXL1* exon 12 mutants lead to a detectable carboxy-terminally truncated protein lacking the plant homology domain (PHD) (Inoue et al. 2016).

Clinical Data

ASXL1 mutations are more common in PMF (18%–37%) and post-MPN AML (17%–47%) compared with early-stage MPNs (Table 1) and are associated with poor prognosis in PV and PMF patients (Vannucchi et al. 2013; Guglielmelli et al. 2014; Tefferi et al. 2014, 2016b, 2018b; Lasho et al. 2018). A worse prognosis for *ASXL1* mutated patients was also described for PMF patients undergoing allogeneic HSC transplantation (Kröger et al. 2017). Monitoring for acquisition of *ASXL1* mutations may be relevant in MPN management, because *ASXL1* mutations are the most frequent mutation acquired during ruxolitinib treatment and are associated with the development of leukocytosis and thrombocytopenia (Newberry et al. 2017).

Mouse Models and Affected Cellular Pathways

ASXL1 mutants missing the PHD domain may not interact with EZH2 (Bera et al. 2012), a member of the PRC2 complex. Consistent with this, ASXL1 mutations modeled by Asxl1 knockdown/knockout contribute to leukemogenesis through derepression of PRC2 targets, such as the Hoxa locus (Fig. 3) (Abdel-Wahab et al. 2012). In an MPN mouse model, *Asxl1* heterozygous knockout cooperated with $JAK2^{V617F}$ to accelerate myelofibrosis and caused leukemia in several mice (Guo et al. 2019). These data show that loss of *ASXL1* promotes MPN progression.

To gain insight into mutant *Asxl1* as opposed to loss of *Asxl1*, multiple groups have generated *Asxl1* knock-in mice (Hsu et al. 2017; Nagase et al. 2018; Yang et al. 2018; Uni et al. 2019). Several mechanisms have been proposed regarding the effects of mutant ASXL1 on PRC1 and on H2AK119ub (the repressive histone mark that PRC1 deposits) (Asada et al. 2018; Nagase et al. 2018; Uni et al. 2019). In contrast to wild-type ASXL1, mutant ASXL1 binds to the

PRC1 member, BMI1 (Uni et al. 2019). A well-known target of BMI1/PRC1 is the p16Ink4a locus, and Ink4a expression is increased in *Asxl1*-mutant knock-in mice, as a result of inhibition of PRC1 by mutant Asxl1 (Uni et al. 2019). Knockout of p16Ink4a in *Asxl1*-mutant knock-in mice rescued the decrease in HSC number and increased apoptosis observed in *Asxl1*-mutant mice, indicating that p16Ink4a plays a key role in *Asxl1*-mutant-driven leukemogenesis (Uni et al. 2019). Another mechanism by which mutant ASXL1 affects H2AK119ub is through the de-ubiquitinase (DUB), BAP1. Although mutant ASXL1 interacts less strongly with BAP1 than wild-type ASXL1 (Abdel-Wahab et al. 2012), it has been shown that mutant ASXL1, but not wild-type ASXL1, enhances the catalytic function of the de-ubiquitinase BAP1. This causes a reduction in global H2AK119ub and locally at HOXA and IRF8 loci in HSCs (Scheuermann et al. 2010; Asada et al. 2018). Depletion of *BAP1* abrogated mutant ASXL1-induced leukemogenesis, showing that also BAP1 plays a crucial role in *Asxl1*-mutant driven leukemia (Asada et al. 2018).

Mutant ASXL1 has been shown to interact with the acetyl lysine reader bromodomain-containing protein 4 (BRD4) (Yang et al. 2018), which plays an important role in gene transcription and regulation through its interaction with pTEFb and superenhancers. Moreover, HSPCs from transgenic *Asxl1*-mutant mice are hypersensitive to bromodomain inhibitors (Yang et al. 2018). Amongst others, one important target gene of mutant ASXL1-BRD4 is *Prdm16* (Yang et al. 2018), which is critical for the maintenance of HSCs. Finally, another mechanism by which mutant ASXL1 has been reported to contribute to leukemogenesis is through H3K and H4K deacetylation mediated repression of TGF-β (Saika et al. 2018).

EZH2

Consequences of Mutations on Gene Function

Enhancer of zeste homolog 2 (EZH2) is a component of PRC2, which harbors the histone lysine *N*-methyltransferase activity of the complex. The most well-studied function of PRC2 is methylation of H3K27 and indeed, H3K27 levels are reduced in *EZH2*-mutant cells (Fig. 3). EZH2 can act as tumor suppressor or oncogene depending on the context. Nonsense, frameshift, and missense mutations have been found in MPNs and are thought to be loss-of-function mutations, suggesting that EZH2 acts as a tumor suppressor in MPNs.

Clinical Data

EZH2 mutations are found in 0%–9% of ET, PV, and PMF patients, and in 13%–15% of post-MPN AML (Table 1). *EZH2* mutations correlate with a worse clinical phenotype with higher leukocyte counts, blast-cell counts, and larger spleens at diagnosis (Guglielmelli et al. 2011). They were shown to be an independent poor prognostic factor (overall survival 31.6 mo vs. wild-type 137 mo) (Guglielmelli et al. 2011).

Mouse Models and Affected Cellular Pathways

Similar to *ASXL1*, *EZH2*-inactivating mutations cause derepression of *HOXA9*, which supports myeloid progenitor cell self-renewal and leukemia development (Khan et al. 2013). In mouse models, concomitant loss of *Ezh2* with expression of $JAK2^{V617F}$ leads to enhanced myelofibrosis compared with $JAK2^{V617F}$ mice, impaired erythroid differentiation, expansion of HSPCs and megakaryocyte progenitors, increased thrombocytosis, and leukocytosis (Shimizu et al. 2016; Yang et al. 2016). However, $JAK2^{V617F}$/*Ezh2* *N*-methyltransferase$^{-/-}$ comutant mice did not develop leukemia, suggesting that additional hits are required for leukemic transformation of $JAK2^{V617F}$ mice.

RNA SPLICING

The third class of mutations comprises mutations in genes involved in RNA splicing (Fig. 3). Two types of spliceosomes exist: the major (U2) and minor (U12) spliceosome. The minor spliceosome splices <1% of all introns, but these are present (alongside U2-type introns) in 700–800

genes, and are evolutionarily conserved (Verma et al. 2018). Mutations in RNA splicing factors SF3B1, U2AF1, SRSF2, ZRSR2, SF3A1, PRPF40B, U2AF2, and SF1 have been reported in myeloid malignancies; the first four are most common and will be discussed below. Spliceosomal mutations are typically mutually exclusive with each other, are relatively rare in PV and ET, and occur at higher frequency in PMF (Grinfeld et al. 2018).

SRSF2

Consequences of Mutations on Gene Function

The most frequent splicing factor mutations in MPNs are found in serine and arginine rich splicing factor 2 (SRSF2), almost all affecting the hotspot proline at position 95. SRSF2 is involved in recognition of exon splicing enhancers. Whereas wild-type SRSF2 binds the RNA sequences GGNG and CCNG in exon splicing enhancers equally well, P95-mutated SRSF2 preferentially splices exons containing the CCNG sequence (Kim et al. 2015).

Clinical Data

SRSF2 mutations are infrequent in ET and PV, whereas they occur in 8%–22% of PMF and post-MPN AML (Table 1). Mutations in *SRSF2* have consistently been associated with poor prognosis in myeloid malignancies including several types of MPN and post-MPN AML, and have been associated with leukemic transformation (Lasho et al. 2012; Zhang et al. 2012; Vannucchi et al. 2013; Tefferi et al. 2018c,d; Venton et al. 2018).

Mouse Models and Affected Cellular Pathways

Mutations in *SRSF2* cause differential splicing of all classes of splicing events (Kim et al. 2015). Examples include missplicing and nonsense-mediated RNA decay-associated down-regulation of EZH2 (Kim et al. 2015) and missplicing of CASP8 causing expression of a truncated CASP8 protein that activates NF-κB signaling (Lee et al. 2018). Expression of mutant SRSF2 causes accumulation of R loops, replication stress, and activation of the ATR-Chk1 pathway (Chen et al. 2018; Nguyen et al. 2018). Wild-type SRSF2 is able to extract RNA polymerase II carboxy-terminal domain (CTD) kinase from the 7SK complex, which causes transcription pause release (Ji et al. 2013). Mutant SRSF2 loses this ability (Chen et al. 2018), which could explain the accumulation of R loops. Mutant SRSF2 also affects transcriptional regulation through predominant splicing of RUNX1 to form the short RUNX1a transcript over the longer RUNX1b transcript (Sakurai et al. 2017). Besides splicing and transcription, SRSF2 plays a role in regulating DNA stability (Xiao et al. 2007). Consistent with this, whole-exome sequencing of patient samples from different myeloid malignancies showed that *SRSF2*-mutant samples have more mutations than samples harboring other spliceosome mutations (Yoshida et al. 2011). Data on the role of *SRSF2* mutations in MPN pathogenesis and on cooperativity with JAK-STAT activating mutations are needed to better delineate their role in MPNs.

U2AF1

Consequences of Mutations on Gene Function

U2 small nuclear RNA auxiliary factor 1 (U2AF1) recognizes the 3′ splice site. U2AF1 contains two mutational hotspot regions (S34 and Q157) located in its two CCCH zinc fingers that are required for RNA binding (Webb and Wise 2004). These mutations alter the preferred −3 (C/A >> T, S34F/Y) or +1 (G >> A, Q157P) nucleotides flanking the AG of the 3′ splice site (Ilagan et al. 2015).

Clinical Data

U2AF1 mutations occur in 16% of PMF and 5%–6% of post-MPN AML patients (Table 1). As for *SF3B1*, PV and ET patients harboring *U2AF1* mutations have an inferior myelofibrosis-free survival compared with *U2AF1* wild-type patients (Tefferi et al. 2016b). Roughly

35% of U2AF1 mutations affect S34 and 65% affect Q157 or its vicinities (Tefferi et al. 2018a). Only Q157 (and near vicinity) mutations are associated with significantly shorter overall survival in MPNs (Tefferi et al. 2018a).

Mouse Models and Affected Cellular Pathways

The prognostic difference between S34 and Q157 mutations could be caused by distinct downstream effects. Indeed, besides very few common targets, U2AF1 S34- and Q157-mutant-expressing blood/MDS cells have mostly distinct expression and splicing patterns, with Q157 mutants causing missplicing of, for example, ARID2 and EZH2 (Przychodzen et al. 2013; Ilagan et al. 2015; Park et al. 2016). Like *SRSF2* mutations, $U2AF1^{S34}$ mutations cause accumulation of R loops (Q157 mutations not investigated) (Nguyen et al. 2018). Besides this canonical function, U2AF1 has been reported to bind mRNA in the cytoplasm and repress mRNA translation (Palangat et al. 2018). The S34F mutation was suggested to affect translation of hundreds of mRNAs. Whether Q157 mutations affect mRNA translation is currently unknown.

ZRSR2

Consequences of Mutations on Gene Function

Zinc finger CCCH-type, RNA binding motif, and serine/arginine-rich 2 (ZRSR2) forms a heterodimer with U2AF2 and is involved in recognizing the 3′ splice site in minor (U12-type) introns, similar to U2AF1 in the major (U2-type) spliceosome. Besides the minor spliceosome, ZRSR2 interacts with major spliceosome components SRSF1/SRSF2 and affects splicing of major introns in vitro, suggesting it also influences the major spliceosome (Shen et al. 2010) and/or other functions of these interaction partners. *ZRSR2* is located on the X chromosome; mutations predominantly occur in males and are frameshift and nonsense mutations in the majority of cases. These data indicate that *ZRSR2* mutations are loss-of-function mutations.

Clinical Data

MPN patients harboring *ZRSR2* mutations are more likely to be diagnosed with PMF (Tefferi et al. 2016a) versus ET or PV (Tefferi et al. 2016b; Grinfeld et al. 2018), suggesting that *ZRSR2* mutations promote myelofibrotic transformation.

Mouse Models and Affected Cellular Pathways

In line with a loss-of-function hypothesis and an important role for *ZRSR2* in the minor spliceosome, mutations in *ZRSR2* predominantly affect splicing of the majority of minor introns, causing intron retention (Madan et al. 2015). Pathways enriched for altered splicing in *ZRSR2*-mutant MDS cells are enriched for MAPK, ErbB signaling, and genes associated with CML and AML (Madan et al. 2015).

SF3B1

Consequences of Mutations on Gene Function

Splicing factor 3b subunit 1 (SF3B1) is part of both the major and minor spliceosome in which it recognizes the BPS as part of the U2 or U11/U12 snRNPs, respectively. Most mutations are found in exons 14–16, position 700 in particular (K700E). SF3B1 mutations cause alternative 3′ splice site selection.

Clinical Data

SF3B1 mutations occur in ~9%–10% of PMF and 4%–7% post-MPN AML patients (Table 1). In the context of MPN, *SF3B1* mutations most commonly occur in the entity MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T). This MDS/MPN overlap syndrome is typically characterized by the presence of both a *JAK2* and *SF3B1* mutation (Jeromin et al. 2013).

Mouse Models and Affected Cellular Pathways

As in other cancers (Darman et al. 2015; DeBoever et al. 2015; Alsafadi et al. 2016), *SF3B1*-

mutated MDS samples mainly had alternative 3′ splice site selection, in many cases leading to nonsense-mediated RNA decay (Lee et al. 2018). Differentially expressed genes included genes involved in RNA processing and metabolism, cell cycle, heme metabolism, and nonsense-mediated decay.

SIGNALING

LNK

Consequences of Mutations on Gene Function

The lymphocyte adaptor protein (LNK) or SH2B adapter protein 3 (SH2B3) is an adaptor protein. It interacts with and inhibits signaling through cytokine and tyrosine kinase receptors such as the EPO receptor and the stem cell factor receptor, c-Kit, or kinases like JAK2 (Fig. 3) (Tong et al. 2005; Bersenev et al. 2008; Simon et al. 2008). LNK inhibits the proliferation of HSCs, B-lymphoid cells, and myeloid cells mainly from the erythroid and megakaryocytic lineage (Takaki et al. 2000, 2002; Velazquez et al. 2002). Negative-feedback loops on growth stimulation are perturbed owing to mutations in *LNK* (and CBL, see below) (Takaki et al. 2002). Many mutations are missense substitutions and target the pleckstrin homology (PH) domain in the majority (~50%) of cases.

Clinical Data

LNK mutations are harbored in 0%–9% of MPN patients and 11% post-AML patients (Table 1). A reduced overall survival has been reported for ET patients harboring *LNK* mutations (Tefferi et al. 2016b).

Mouse Models and Affected Cellular Pathways

Mice lacking *Lnk* developed several characteristics of MPNs, including extramedullary hematopoiesis and splenomegaly, and an increase in HSCs and myeloid progenitor cells, predominantly of the megakaryocytic lineage (Velazquez et al. 2002; Ema et al. 2005; Buza-Vidas et al. 2006; Seita et al. 2007; Bersenev et al. 2008; Takizawa et al. 2008). The SH2 domain of LNK binds nonphosphorylated and JH2 domain-phosphorylated JAK2, thereby inhibiting JAK2 activation (Kurzer et al. 2006; Bersenev et al. 2008; Gery et al. 2009; Baran-Marszak et al. 2010). Additionally, LNK blocks binding of activators with c-Kit and activation of downstream signaling (Takaki et al. 2002; Simon et al. 2008). In addition, LNK can recruit the E3 ubiquitin ligase CBL for degradation of receptors/signaling molecules. These negative-feedback loops on growth stimulation are perturbed owing to mutations in *LNK* (Takaki et al. 2002). *LNK* mutations have been shown to enhance growth of $JAK2^{V617F}$ cells in clonogenic assays and in mice (Bersenev et al. 2008; Gery et al. 2009).

CBL

Consequences of Mutations on Gene Function

Casitas B-cell lymphoma (CBL) is a RING E3 ubiquitin ligase that promotes K63- and K48-linked ubiquitination (Thien and Langdon 2005; Mohapatra et al. 2013). It recognizes activated (receptor) tyrosine kinases such as epidermal growth factor receptor (EGFR), c-Kit, FLT3, and JAK2 by their phosphorylation and ubiquitinates them, which leads to proteasomal degradation (Thien and Langdon 2005; Mohapatra et al. 2013; Lv et al. 2017). Mutations are mostly homozygous missense substitutions located in the RING and linker domain, significantly reducing the E3 ligase activity, and thus decreasing the degradation of its substrates.

Clinical Data

Mutations in CBL are rare in ET and PV, but occur in up to 6% of MF and 4% of post-MPN AML patients (Table 1).

Mouse Models and Affected Cellular Pathways

CBL mutations lead to increased STAT5 and Akt phosphorylation, cytokine hypersensitivity, and cell proliferation (Sanada et al. 2009). *CBL* mu-

tations are not merely loss-of-function mutations, because they further augmented cytokine sensitivity found in CBL knockout cells (Sanada et al. 2009).

NRAS/KRAS

Consequences of Mutations on Gene Function

Heterozygous missense substitutions at NRAS/KRAS codons 12, 13, and 61 are most common and favor the GTP-bound state of RAS because of reduced intrinsic GTP hydrolysis and resistance to GAPs (Schubbert et al. 2007). These mutations cause constitutive activation of growth signaling.

Clinical Data

Mutations in NRAS and KRAS in MPNs are associated with leukemic transformation and have been found in 7%–15% of post-MPN AML patients (Table 1), mutations in NRAS being more frequent than in KRAS.

Mouse Models and Affected Cellular Pathways

Conditional expression of $Kras^{G12D}$ in mouse models causes a myeloproliferative disease, whereas the same mutation in Nras causes a much more indolent disease (MacKenzie et al. 1999; Chan and Gilliland 2004).

PTPN11

Consequences of Mutations on Gene Function

Protein tyrosine phosphatase, nonreceptor type 11 (PTPN11) is a protein tyrosine phosphatase that acts downstream from several transmembrane receptors and dephosphorylates RAS (Bunda et al. 2015). Mutations in AML target the amino-terminal Src-homology 2 (N-SH2) and phosphotyrosine phosphatase (PTP) domains, which are important for switching between inactive and active forms of PTPN11.

Clinical Data

Heterozygous missense mutations in *PTPN11* are found in 6%–8% of post-MPN AML cases (Table 1) and are associated with shortened survival (Lasho et al. 2018).

Mouse Models and Affected Cellular Pathways

PTPN11 mutations cause a very high phosphatase activity (Tartaglia et al. 2003, 2006; Keilhack et al. 2005; Niihori et al. 2005). Dephosphorylation of RAS increases RAF–RAS association and activation of the RAS–RAF–MEK–ERK pathway (Shi et al. 2000).

TRANSCRIPTION FACTORS

RUNX1

Consequences of Mutations on Gene Function

Runt-related transcription factor 1 (RUNX1) heterodimerizes with core binding factor β (CBFβ) and regulates key hematopoietic transcriptional programs. It contains a DNA-binding and nuclear localizing Runt homology domain (RHD). Missense, frameshift, and nonsense mutations inactivate the affected RUNX1 protein, and, in addition, mutant RUNX1 may act in a dominant-negative way over the remaining wild-type RUNX1 protein.

Clinical Data

RUNX1 mutations occur in 4%–13% of post-MPN AML patients (Table 1). Multivariate analysis showed significantly shortened survival for *RUNX1* mutated versus *RUNX1* wild-type post-MPN AML patients (Lasho et al. 2018; McNamara et al. 2018).

Mouse Models and Affected Cellular Pathways

RUNX1 inactivation contributes to AML development through reduced myeloid differentiation and increased HSC self-renewal. The most frequent dominant-negative mutation, D171N, is located in its DNA-binding RHD. Mice trans-

planted with bone marrow cells overexpressing RUNX1^{D171N} developed MDS and MDS/AML within 4–13 mo after transplantation (Watanabe-Okochi et al. 2008). Ectopic expression of RUNX1^{D171N} in CD34^{+} HSPCs from chronic phase MPN patients seemed to keep the cells in a HSPC state and increase proliferation (Ding et al. 2009). Besides mutations in RUNX1, the short RUNX1a isoform has been shown to be overexpressed in MPN (Sakurai et al. 2017). As is the case for many leukemia-associated RUNX1 mutations, RUNX1a has been proposed to act in a dominant-negative way over the other two isoforms RUXN1b and RUNX1c. RUNX1a expression causes expansion of murine HSCs and up-regulates *Hoxa9*, *Meis1*, and *Stat1* (Tsuzuki and Seto 2012; Ran et al. 2013).

NFE2

Consequences of Mutations on Gene Function

A 4-amino acid in-frame deletion and frameshift mutations leading to expression of a carboxy-terminally truncated protein have been found in the transcription factor nuclear factor, erythroid 2 (NFE2) in MPNs. The mutations caused increased mRNA and protein expression of wild-type NFE2 (Jutzi et al. 2013).

Clinical Data

Mutations in NFE2 are carried by 0%–3% of PV and MF patients (Table 1).

Mouse Models and Affected Cellular Pathways

Mice transplanted with ectopically expressing mutant NF-E2 bone marrow cells had elevated myeloid progenitor cells as well as platelet numbers, erythrocyte numbers, and absolute neutrophil counts compared with control mice (Jutzi et al. 2013). When combined with JAK2^{V617F}, JAK2^{V617F}/NFE2 comutant mice had elevated hemoglobin and white blood cell count compared with JAK2^{V617F} mice (Jutzi et al. 2013). These data show that mutant NFE2 promotes myelopoiesis and augments the JAK2^{V617F}-induced MPN phenotype. Besides mutated NFE2, increased expression of NFE2 has been found in MPN patient samples. One of the proposed mechanisms for elevated NFE2 expression includes JAK2-dependent phosphorylation of H3Y41 in the *NFE2* promoter, leading to decreased binding of the repressive HP1α protein (Peeken et al. 2018). Increased NFE2 expression causes elevated transcription of the histone demethylase JMJD1C. In turn, JMJD1C causes positive feedback through decreasing H3K9me2 on the NFE2 promoter, also reducing HP1α binding and increasing NFE2 expression (Fig. 3) (Peeken et al. 2018).

DNA DAMAGE RESPONSE/STRESS SIGNALING

TP53

Consequences of Mutations on Gene Function

The last class of genes found mutated in MPNs includes genes involved in the DNA damage response and cellular stress. Tumor protein P53 (TP53) is a transcription factor frequently mutated in cancer. It is essential for a proper cellular response to stress and DNA damage. Many *TP53* mutations are missense mutations, which cause an abundance of mutant TP53 protein compared with wild-type TP53 (Rotter 1983). TP53 is a tumor suppressor, and *TP53* mutations can have several (nonmutually exclusive) effects: (1) loss of tumor suppressor function, (2) dominant-negative effect on wild-type TP53 protein, and (3) gain of functions that the wild-type protein does not possess.

Clinical Data

Mutations in *TP53* are uncommon in chronic phase MPNs, but exist in 11%–36% of post-MPN AML patients (Table 1). In addition to higher mutational frequency, the variant allele fraction of mutant *TP53* is higher in patient samples from transformed patients compared with chronic phase samples (Rampal et al. 2014). These data suggest that *TP53* mutations contribute to leukemic transformation in MPN.

Post-MPN AML patients harboring *TP53* mutations have an inferior overall survival compared with wild-type patients (mutant 4.4 mo vs. wild-type 6.5 mo) (Lundberg et al. 2014; Venton et al. 2018).

Mouse Models and Affected Cellular Pathways

In line with patient data, the concomitant knockout of *Tp53* and expression of $JAK2^{V617F}$ leads to leukemic transformation in mouse models (Rampal et al. 2014). Several progenitor populations from these mice were able to induce leukemia in (secondary) recipients. It has been shown that *TP53* mutations contribute to AML by increasing HSC self-renewal and resistance to cellular stress (Liu et al. 2009).

PPM1D

Consequences of Mutations on Gene Function

The serine-threonine *Protein Phosphatase* $Mg2^+/Mn2^+$ *1D* (*PPM1D*) gene is transcriptionally upregulated on *TP53* induction in response to DNA damage (Fiscella et al. 1997) and in turn negatively regulates TP53 and other proteins involved in the DNA damage response. Truncating and frameshift *PPM1D* mutations in exon 6 have been found in clonal hematopoiesis, MDS, and MPNs (Genovese et al. 2014; Xie et al. 2014; Coombs et al. 2017; Lindsley et al. 2017).

Clinical Data

PPM1D was recently described to be mutated in MPNs, with 1.9% of patients affected (Table 1; Grinfeld et al. 2018). Interestingly, *PPM1D* mutations are more frequent in patients who have been exposed to chemotherapy and who were diagnosed with therapy-related myeloid neoplasms (Ruark et al. 2013; Zhang et al. 2014; Kahn et al. 2018).

Mouse Models and Affected Cellular Pathways

PPM1D exon 6 mutations cause expression of a truncated PPM1D protein that lacks a carboxy-terminal degradation domain (Kahn et al. 2018). Consistent with patient data, *PPM1D*-mutant cells are selected for when treated with chemotherapy (Hsu et al. 2018; Kahn et al. 2018). This is explained by an abrogated DNA damage response, causing altered cell cycle progression, decreased apoptosis, and reduced mitochondrial priming in *PPM1D*-mutant compared with control cells (Kahn et al. 2018).

THERAPEUTIC IMPLICATIONS

Although the molecular pathogenesis of MPNs has been comprehensively defined, there are currently no curative pharmacological treatment options. The MPN phenotypic driver mutations represent attractive therapeutic targets because they are disease-initiating, activating, and can occur as the sole mutation, particularly in PV and ET. The main deficiency of JAK2 inhibitors is an absence of clonal selectivity, and efforts are ongoing to develop JAK2V617F-mutant-specific inhibitors. Mutant CALR also represents an enticing therapeutic target in MPNs, particularly from an immunological perspective, given its shared mutant-specific carboxy-terminal peptide.

Almost all of the concomitant mutations seen in MPNs do not currently have rationally designed approaches to target them, with IDH1/2 being the rare exception. Two drugs that inhibit mutant IDH1 or mutant IDH2 have been approved for the treatment of adult refractory or relapsed AML in the past 2 years: ivosidemib and enasidenib, respectively. Because mutations in IDH proteins are associated with a high risk of leukemic transformation in MPN, this is an important clinical development. Spliceosomal mutations, in particular *SRSF2* mutations, are another class of mutations associated with disease progression in MPNs, and there has been great enthusiasm around the development of spliceosomal inhibitors in myeloid malignancies more broadly. However, early clinical trial data in myelodysplastic syndrome (MDS) indicates an absence of clonal selectivity for spliceosomal inhibitors, although further studies are needed.

Another challenge posed by the genomic complexity of MPNs is that even drugs with

potent, on-target, mutant-specific activity may have limited clinical efficacy depending on the clonal architecture of the MPNs (e.g., targeting a subclonal mutation as compared with a truncal lesion). Treatment paradigms that invoke early intervention to target disease-initiating genetic events and therapeutic approaches with broad efficacy regardless of molecular genetics (e.g., immunotherapy) are attractive for this reason.

CONCLUDING REMARKS

MPN phenotypic driver mutations that activate JAK-STAT signaling are central to MPN pathogenesis. However, concomitant somatic mutations are common and are often associated with disease progression. With the integration of NGS panels into MPN clinical care, molecular genetics is increasingly being used to predict prognosis and estimate the risk of disease progression (e.g., to AML) (Grinfeld et al. 2018). In myelofibrosis, clinical prognostic models that include molecular genetic parameters have already been developed (Ciboddo and Mullally 2018). The prognostic significance of concomitant somatic mutations in ET and PV is starting to be explored (Tefferi et al. 2016b; Grinfeld et al. 2018). With these advances, we have seen the development of novel mouse models to study the effects of concomitant mutations on MPN stem cells (Jacquelin et al. 2018; McKenney et al. 2018b) and identify the mechanisms by which these mutations drive disease progression. The next step is to use this knowledge to develop novel treatment approaches that exploit molecular vulnerabilities and preferentially target MPN stem cells in patients.

COMPETING INTEREST STATEMENT

The authors have declared that no conflict of interest exists.

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