EUROPEAN HEMATOLOGY ASSOCIATION

Myeloproliferative neoplasms - Section 1

Molecular genetics in negative myeloproliferative neoplasms

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Take-home messages

- Mutational profiling of MPN should include driver mutations and, in selected cases, additional mutations in genes associated more generally with myeloid malignances; in about 10-15% of the patients, however, no driver mutation is detected ('triple negative').
- Driver mutations represent major diagnostic criteria for MPN in the revised 2016 WHO classification and, therefore, these should be determined in each patient.
- Both driver and additional non-driver mutations are associated with phenotypic traits and, importantly, they may contribute to estimate prognosis.

Introduction

The classic Philadelphia chromosome negative myeloproliferative neoplasms (MPN), that include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), originate from a single hematopoietic stem cell (HSC) that, having somatically acquired one or more mutations, expands and produces a progeny of terminally differentiated myeloid cells of different lineages as well as, at least in some cases, B and NK cells.[1-3] Phenotypically, MPN are very akin in terms of clinical presentation: thrombosis, hemorrhage, burdensome systemic manifestations, splenomegaly, progression to secondary forms of myelofibrosis (in case of PV and ET) and acute leukemia or myelodysplasia, represent the main clinical events occurring over the course of the disease, and may eventually lead to death.[4] On the other hand, the clonal expansion of mutated HSC may manifest with either prevalent multilineage (PV, MF) or unilineage hyperplasia (ET); the genetic mechanisms that contribute to the uniqueness of one versus another form of MPN represent one of the major unsolved issues in the understanding of molecular pathogenesis of MPN. In fact, no unique phenotype-restricted single disease-associated mutation has been discovered. Rather, MPN are characterized by a restricted set of largely overlapping driver mutations, that include the *JAK2*V617F mutation (found in >95% of PV and 60% of ET and PMF), *MPL* mutations, mainly at codon 515 (3-8% in ET and PMF, rare atypical *MPL* mutations also exist, including S505N mutation initially reported as germline mutation), calreticulin mutations (*CALR*; 20-25% in ET and PMF), and also mutations of *JAK2* exon 12 in 3% of *JAK2*V617F-negative PV. [5,6] Non-canonical mutations in *JAK2* and *MPL* have been reported in a minority of 'triple-negative' (TN) patients with ET and PMF, as these patients have operationally been defined by the lack of known driver mutations that currently comprise 10-15% of ET and PMF cases.[9] It is also likely that some cases currently defined as ET are not true clonal disorders, but might include uncharacterized familial variants. When expressed in the mouse, the above driver mutations reproduce an MPN-like disorder, with some differences among models due to the experimental setting and, possibly, the extent of intracellular JAK2 signaling.[10] In fact, abnormal activation of the JAK/STAT signaling pathway is a shared characteristic of the above mentioned driver mutations, and this finding further complicates the understanding of mutation-phenotype correlates. On the other hand, the uniform activation of JAK2 signaling in MPNs helps to explain the reported clinical efficacy of JAK1/2 inhibitors, for which the underlying mutation status is largely irrelevant.

Many patients with MPN (about 20-50% of ET and PV, and even more in PMF) harbor additional mutations (listed in Table 1) that are non-exclusive of MPN, since they are found also in acute leukemia or myelodysplasia, and do not show any selective clustering with driver mutations. The most common mutations target regulators of DNA methylation (*TET2, DNMT3A*), histone modifiers (members of the Polycomb repressor complex 1 & 2, IDH1/2), components of the splicea-

some (*SF3B1, SRSF2*), transcription factors (TP53, CUX1, Ikaros), *bona fide* oncogenes (*NRAS, KRAS*) and proteins involved in signaling (*LNK, CBL*), to name the most frequently involved genes. These mutations usually occur as subclonal events, often with low variant allele frequency and, therefore, their role in disease initiation is difficult to assess. However, they have been associated with important clinical end-points (survival, rate of leukemia transformation).[11,12] Often, these subclonal mutations occur as multiple events, reflecting an intrinsic genetic complexity of the disease and likely expressing a higher propensity to clonal progression. This might contribute to their prognostically negative impact;[11] indeed, patients with PMF have multiple mutations more commonly than patients with PV and ET.[12] The interpretation of the above

Table 1. List of the most frequently detected mutations in patients with myeloproliferative neoplasms.

	Localization	Function	Type of abnormalities
Signaling			
JAK2ex14	9p24	Tyrosine Kinase, signaling	Gain of function
JAK2ex12	9p24	Tyrosine Kinase, signaling	Gain of function
MPL	1p34	Receptor, signaling	Gain of function
CALR	19p13	ER-associated multifunction protein	Gain of function through wild-type MPL
SH2B3(LNK)	12q24	Adaptor, signaling	Loss of function
CBL	11q23	Adaptor, E3 ubiquitin ligase, signaling	Dominant negative
SOCS1	16p13.2	E3 ubiquitin ligase, signaling	Methylation
SOCS2	12q22	E3 ubiquitin ligase, signaling	Methylation
SOCS3	17q25.3	E3 ubiquitin ligase, signaling	Methylation, mutation
Epigenetic			
TET2	4q24	DNA hydroxymethylation	Loss of function
ASXL1	20q11.21	Chromatin modifications	Loss of function
EZH2	7q35	Chromatin methylation	Loss of function
JARID	6p24	Chromatin methylation	Loss of function
SUZ12	17q11.2	Chromatin methylation	Loss of function
IDH1	2q33.3	Metabolism	Neomorphic enzyme
IDH2	15q26.1	Metabolism	Neomorphic enzyme
EED	11q14.2	Chromatin methylation	Loss of function
Splicing			
SRSF2	17q25.1	Spliceasome	Loss of function
U2AF1	21q22.3	Spliceasome	Loss of function
ZRSR2	7q25.1	Spliceasome	Loss of function
SF3B1	2q33.1	Spliceasome	Loss of function
Leukemia progression			
TP53	17p13.1	Cell cycle, apoptosis	Loss of function
SMD4	1q32	TP53 regulator	Amplification
DNMT3A	2p23	Chromatin modifications	Loss of function
RB	13q14	Cell cycle, apoptosis	Deletion
IKZF1	7p12	Transcription factor	Deletion
RUNX1	21q22.3	Transcription factor	Loss of function
NRAS	1p13.2	GTPase, signaling	Gain of function

findings is further complicated by the discovery of the phenomenon of clonal hematopoiesis of indeterminate potential (CHIP), reflecting the acquisition of similar mutation profiles as an age-dependent phenomenon in the otherwise healthy population.[13] The two most frequent mutated genes in the settings of CHIP are *TET2* and *DNMT3A*; it is hypothesized that by increasing the self-renewal capability of HSC, these mutated genes might favor the acquisition of secondary mutations eventually resulting in clonal predominance. Recently, evidence has also been provided that the order in which driver mutations are acquired on the background of other somatic mutations affects disease presentation.[14]

Little is known about the genetic events that promote transformation of a chronic disease to acute leukemia; these aggressive secondary leukemias do not display, if rarely at all, the typical genetic abnormalities found in *de novo* leukemia (such as FLT3, NPM1), but are enriched in mutations of the *TP53* gene, including missense mutations or deletions.[15] Also, amplification of the locus of MDM4, a TP53 transcriptional inhibitor, has been reported as a mechanism of functional insufficiency of TP53. Clones harboring mutated *TP53* may sporadically be found even in the early chronic phase of MPN, thus they are not strictly predictive of the risk to develop leukemia in the individual patient; rather, the transition from mutated *TP53* heterozygosity to homozygosity likely represents one key mechanism responsible for the development of leukemia.

Analysis and characterization of driver and non-driver mutations currently deserves a remarkable role for diagnosis and has increasing relevance for prognostication in patients with MPN. Driver mutations are listed as major diagnostic criteria in the revised 2016 World Health Organization classification,[16] while the detection of non-driver mutations may support evidence of clonal hematopoiesis existing in patients who present a hematologic phenotype suggestive of an underlying MPN but lack driver mutations. Furthermore, there are phenotypic and prognostic correlates with driver mutations (for example the lower risk of thrombosis in *CALR* mutated patients with ET compared to the *JAK2*V617F/*MPL* mutated ones[17] or the negative impact of triple negativity on survival in PMF[18]) as well as with non-driver mutations (the adverse prognostic significance of the so called 'high mutation risk' (HMR) phenotype, represented by mutations in *ASXL1, EZH2, SRSF2, IDH1/2* in PMF[19]). Further efforts are required in order to integrate all of this information with already validated clinical and hematologic variables, and possibly with other genetic phenotype modifiers, in order to obtain a full picture of the epidemiology, clinical significance and pathogenetic role of such a complex genetic background; this knowledge might be key to facilitate identification of novel targets for therapy.[20]

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