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EDITORIAL

Mutations galore in myeloproliferative neoplasms: Would the real Spartacus please stand up?

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Current evidence supports the view that *BCR-ABL1* is the disease-causing mutation in chronic myelogenous leukemia (CML): (i) it is invariably present in CML and absent in other chronic myeloid malignancies;[1] (ii) it induces CML-like disease in mice;[2] and (iii) anti-BCR-ABL1 targeted therapy in CML results in complete and durable hematologic, cytogenetic and molecular remissions.[3] In an ongoing quest to identify *BCR-ABL1*-like mutations in other myeloproliferative neoplasms (MPNs), scientists have encountered an apparently endless number of mutations involving a spectrum of genes, including *JAK2*, *MPL*, *LNK*, *CBL*, *IKZF1*, *IDH1*, *IDH2*, *TET2*, *ASXL1* and *EZH2* (Table 1).[4] In the current issue of *Leukemia*, three papers[5–7] report on yet another gene, *DNMT3A*, which is affected by somatic mutations in both *BCR-ABL1*-negative MPN and myelodysplastic syndromes (MDSs).

DNMT3A, DNMT3B and DNMT1 are DNA cytosine methyltransferases that are essential in establishing and maintaining DNA methylation patterns in mammals.[8,9] Germline *DNMT3B* mutations have been associated with the autosomal recessive immunodeficiency, centromere instability and facial anomalies (ICF) syndrome.[10,11] Heterozygous *DNMT3A*R882 mutations were first reported in acute myeloid leukemia (AML) by Yamashita *et al.*[12] in 3 (4%) of 74 patients. Subsequently, Ley *et al.*[13] described *DNMT3A*R882 (60% of mutant *DNMT3A* cases) and other heterozygous *DNMT3A* mutations in 62 (~22%) of 281 AML patients. In the latter study, *DNMT3A* mutations were enriched for older patients, normal karyotype and mutations involving *FLT3*, *NPM1* and *IDH1*. Multivariable analysis identified mutant *DNMT3A*, older age and *FLT3* mutations as risk factors for survival, but the particular observation was confounded by the inclusion of patients with favorable cytogenetic and molecular (*NPM1*-mutated/*FLT3*-unmutated) profiles; *DNMT3A* mutations were mutually exclusive of the former and did not affect survival in the latter group of patients.[13]

In the current issue of *Leukemia*, Walter *et al.*[7] report on *DNMT3A* mutations in 12 (8%) of 144 patients with MDS (one patient had two *DNMT3A* mutations that made the total 13). As was the case with AML, R882, which is located in the methyltransferase domain, was the most frequent amino acid affected by these mutations (4 of 13 mutations; ~31%). The other mutations were also located in the methyltransferase domain and included P904L ($n=2$), L737R, R771L, S770W, S714C, R635W, Q237X and L442X. *DNMT3A* mutational frequencies in MDS variants were 6% (4 of 67) for refractory anemia (RA), 8% (6 of 72) for RA with excess blasts (RAEB), 20% (1 of 5) for RA with ring sideroblasts (RARS), 10% (7 of 69) for MDS with normal karyotype, 6% (2 of 36) for MDS with complex karyotype and 6% (2 of 34) for MDS with either trisomy 8 or −7/del(7q). In this particular MDS study, sample size was too small and patient population too heterogeneous to properly assess the prognostic value of *DNMT3A* mutations, however, univariate analysis showed worse survival in *DNMT3A*-mutated cases. In another MDS study, Ewalt *et al.*[14] screened 100 patients with RAEB and found four cases (4%) with *DNMT3A*R882 mutations, one of which also harbored a second *DNMT3A* mutation (*DNMT3A*C709Y).

Also in the current issue of *Leukemia*, two papers report on *DNMT3A* mutations in patients with MPN. Stegelmann *et al.*[6] studied 30 patients each with essential thrombocythemia or polycythemia vera, 16 with primary myelofibrosis, 4 with post-thrombocythemia/polycythemia vera MF and 35 with blast phase MPN; the corresponding mutational frequencies were ~0% (0 of 30), 7% (2 of 30), 6% (1 of 16), 50% (2 of 4) and 14% (5 of 35). In the second study, Abdel-Wahab *et al.*[5] studied 46 patients with primary myelofibrosis, 22 with post-thrombocythemia/polycythemia vera MF and 11 with blast phase MPN; *DNMT3A* mutational frequencies were reported at 7, 0 and 0%, respectively. All 13 *DNMT3A* mutations reported in these two studies were heterozygous and included R882H/C ($n=5$), E477 (nonsense), E523 (nonsense), W305 (frameshift), R488 (frameshift), P264 (frameshift), D768 (frameshift), G120 (frameshift) and P419 (frameshift); additional *DNMT3A* sequence variants (unannotated single nucleotide polymorphisms vs missense mutations) that were reported included N501S, W860R, E30A, P99S, P569A, R659H and R899C. *DNMT3A* mutations in the aforementioned two MPN studies were documented to occur in the presence or absence of *JAK2*, *IDH*, *ASXL1* or *TET2* mutations.

The question now is whether or not we have been enlightened more about the pathogenesis of *BCR-ABL1*-negative MPN, as a result of a growing list of mutations they harbor. Are these mutations all critical drivers of the underlying myeloproliferative process or are some of the mutations simply markers of cancer-associated genomic instability? Which mutations are important for chronic phase disease and which ones contribute to disease transformation into myelofibrosis or AML? Is there more than one critical mutation for a specific disease phenotype? (i.e., Do multiple mutations share a common phenotype?) Alternatively, are we dealing with more than three diseases in the WHO category of *BCR-ABL1*-negative MPN? (i.e., Are we unintentionally lumping molecularly distinct diseases into a single clinicopathologic entity?)

It is becoming increasingly evident that currently known MPN-associated mutations likely represent secondary events, are not necessarily mutually exclusive and do not display a predictable pattern of clonal hierarchy.[15,16] For example, in a recent report, a patient with normal karyotype primary myelofibrosis displayed four distinct mutations including *JAK2*V617F, *IDH2*R140Q and two *LNK* mutations affecting both parental *LNK* alleles;[17] single colony studies were carried out and disclosed one of the two *LNK* mutations as the first and *JAK2*V617F as the last event, in the order of clonal evolution. Others have shown that in patients with concomitant *TET2* and *JAK2* mutations, the two can involve separate clones or one can emerge before or after the other.[15] Furthermore, no one mutation has been directly implicated in leukemic

Table 1 Currently known mutations in BCR-ABL1-negative myeloproliferative neoplasms

Mutations	*Chromosome location*	*Mutational frequency*	*Pathogenetic relevance*
JAK2 (Janus kinase 2) *JAK2V617F* exon 14 mutation[4]	9p24	PV ~ 96% (ref. 4) ET ~ 55% (ref. 4) PMF ~ 65% (ref. 4) BP-MPN ~ 50% (ref. 4)	Contributes to abnormal myeloproliferation and progenitor cell-growth factor hypersensitivity[4]
JAK2 exon 12 mutation[4]	9p24	PV ~ 3% (ref. 4)	Contributes to primarily erythroid myeloproliferation[4]
MPL (Myeloproliferative leukemia virus oncogene) MPN-associated MPL mutations involve exon 10 (ref. 4)	1p34	ET ~ 3% (ref. 4) PMF ~ 10% (ref. 4) BP-MPN ~ 5% (ref. 4)	Contributes to primarily megakaryocytic myeloproliferation[4]
LNK (as in Links) a.k.a. *SH2B3* (a membrane-bound adaptor protein) MPN-associated mutations were monoallelic and involved exon 2 (ref. 22,27)	12q24.12	PV ~ rare[27,43] ET ~ rare[22,27] PMF ~ rare[22,27] BP-MPN ~ 10% (ref. 27)	Wild-type LNK is a negative regulator of JAK2 signaling[44]
TET2 (TET oncogene family member 2) Mutations involve several exons[4,45]	4q24	PV ~ 16% (ref. 4) ET ~ 5% (ref. 4) PMF ~ 17% (ref. 4) BP-MPN ~ 17% (ref. 4) AML ~ 20% (ref. 46) MDS ~ 26% (ref. 47) CMML ~ 51% (ref. 48) SM ~ 29% (ref. 49) RARS-T ~ 26% (ref. 50)	TET proteins catalyze conversion of 5-methylcytosine (5 mC) to 5-hydroxymethylcytosine (5 hmC), which favors demethylated DNA. Both TET1 (ref. 51) and TET2(ref. 32) display this catalytic activity. *IDH* and *TET2* mutations might share a common pathogenetic effect, which might include abnormal DNA hypermethylation and impaired myelopoiesis.
ASXL1 (additional sex combs-like 1) Exon 12 mutations[52]	20q11.1	ET ~ 3% (ref. 53) PMF ~ 13% (ref. 54) BP-MPN ~ 18% (ref. 54) AML ~ 11% (ref. 55) MDS ~ 11% (ref. 52) CMML ~ 43% (ref. 52)	Wild-type ASXL1 is needed for normal hematopoiesis[56] and might be involved in co-activation of transcription factors and transcriptional repression.[57,58]
IDH1/*IDH2* (Isocitrate dehydrogenase) Exon 4 mutations[29]	2q33.3/15q26.1	PV ~ 2% (ref. 29) ET ~ 1% (ref. 29) PMF ~ 4% (ref. 29) BP-MPN ~ 20% (ref. 29) AML ~ 14% (ref. 59) MDS ~ 5% (ref. 30)	*IDH* mutations induce loss of activity for the conversion of isocitrate to 2-ketoglutarate (2-KG) and gain of function in the conversion of 2-KG to 2-hydroxyglutarate (2-HG).[36,37] 2-HG might be the mediator of impaired TET2 function in cells with mutant *IDH* expression.[35]
EZH2 (enhancer of zeste homolog 2) Mutations involve several exons[34]	7q36.1	PV ~ 3% (ref. 34) PMF ~ 7% (ref. 54) MDS ~ 6% (ref. 34,60) CMML ~ 13% (ref. 34) aCML ~ 13% (ref. 34) HES/CEL ~ 3% (ref. 34)	Wild-type EZH2 is part of a histone methyltransferase (polycomb repressive complex 2 associated with H3 Lys-27 trimethylation). MPN-associated *EZH2* mutations might have a tumor suppressor activity,[34] which contrasts with the gain-of-function activity for lymphoma-associated *EZH2* mutations.[33]
DNMT3A (DNA cytosine methyltransferase 3a) Most frequent mutations affect amino acid R882 (ref. 13)	2p23	PV ~ 7% (ref. 6) PMF ~ 7% (ref. 5,6) BP-MPN ~ 14% (ref. 5,6) AML ~ 22% (ref. 13) MDS ~ 8% (ref. 7)	DNA methyl transferases are essential In establishing and maintaining DNA methylation patterns in mammals.[8,9]
CBL (Casitas B-lineage lymphoma proto-oncogene) Exon 8/9 mutations[61]	11q23.3	PV ~ rare[61] ET ~ rare[61] MF ~ 6% (ref. 61)	CBL is an E3 ubiquitin ligase that marks mutant kinases for degradation. Transforming activity requires loss of this function.[62]
IKZF1 (IKAROS family zinc-finger 1) (mostly deletions including intragenic)[63]	7p12	CP-MPN ~ rare[63] BP-MPN ~ 19% (ref. 63)	IKZF1 is a transcription regulator and putative tumor suppressor[64]

Abbreviations: AML, acute myeloid leukemia; BP-MPN, blast phase MPN; aCML, atypical chronic myeloid leukemia, *BCR-ABL1*-negative; CMML, chronic myelomonocytic leukemia; CP-MPN, chronic phase MPN; ET, essential thrombocythemia; HES/CEL, hypereosinophilic syndrome/chronic eosinophilic leukemia; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasms; PMF, primary myelofibrosis; PV, polycythemia vera; RARS-T, refractory anemia with ring sideroblasts; SM, systemic mastocytosis.
MF includes both PMF and post-ET/PV myelofibrosis.

transformation, which can involve either mutated or unmutated clones, in otherwise mutation-positive patients.[18]

Among the currently known MPN mutations, *JAK2*, *MPL* and *LNK* mutations[19–22] are the most noteworthy because: (i) they share similar functional consequences that include constitutive JAK–STAT activation and induction of MPN-like disease in mice;[19,20,22,23] (ii) *JAK2* mutations are by far the most prevalent and occur in virtually all patients with polycythemia vera[24] and in the majority of those with thrombocythemia or primary myelofibrosis;[25] and (iii) all three mutations are relatively specific to MPN.[26,27] However, the fact remains that none of these three mutations can be traced back as the ancestral disease clone and small molecule drugs that target their common effector pathway have not produced clonal remissions.[28] Disease-specific pathogenetic relevance for non-*JAK2* MPN mutations is further undermined by low mutational frequency and promiscuity within the spectrum of myeloid neoplasms (Table 1) (ref. 4). The latter often involve genes that are thought to be epigenetically relevant, including *TET2, ASXL1, IDH, EZH2* and now, *DNMT3A*.[29,30]

Global DNA hypomethylation and regional hypermethylation of promoters for tumor suppressor genes are typical findings in cancer and are believed to contribute to genomic instability.[31] Such epigenetic changes might result from mutations that affect expression or function of DNA methyl transferases or other epigenetically relevant proteins such as TET2, which catalyzes conversion of 5-methylcytosine to 5-hydroxymethylcytosine[32] or EZH2, which is part of a histone methyl transferase complex.[33] Consistent with this view, there is evidence to suggest that *DNMT3A*[12,13] and MPN-associated *EZH2* (ref. 34) mutations result in loss of function. Similarly, loss-of-function mutations involving *TET2* might result in decreased generation of 5-hydroxymethylcytosine and, therefore, dysregulated regional hypermethylation. Furthermore, a recent paper suggested that mutant IDH might mimic the epigenetic effect of mutant TET2, by inhibiting the catalytic activity of wild-type TET2, through generation of 2-hydroxyglutarate.[35–37] Of note, *JAK2* mutations might also have an epigenetic effect, the *in vivo* pathogenetic relevance of which is uncertain.[38,39]

Taken together, it seems that we need to re-examine our pathogenetic concept and treatment paradigm in *BCR-ABL1*-negative MPN. It is possible that we might never find *BCR-ABL1*-like mutations in these diseases, which undermines the prospect of an imatinib-CML-like experience. What we currently have is a laundry list of ‘secondary’ mutations, most of which can be operationally organized into JAK–STAT-relevant and epigenetically relevant mutations. The former (i.e., *JAK2*, *MPL* and *LNK* mutations) seem to be relatively specific to MPN and, in that context, are likely to represent driver mutations. The latter (i.e., *TET2*, *EZH2*, *IDH*, *DNMT3A* and possibly *ASXL1*) might carry a broader, albeit nonspecific, pathogenetic relevance that might include sustenance of the myeloid neoplasm stem cell and clonal evolution. At the same time, we should not be intimidated by the possibility that some mutations in either MPN or MDS might simply represent passenger mutations, regardless of what we think their functional relevance might be.

From a therapeutic standpoint, a better understanding of pathway wiring rather than description of new secondary mutations is more likely to lead to identification of a robust drug target. The big picture also requires consideration of the pathogenetic contribution of the interaction between host and tumor and the phenotype modifying and prognostic impact of abnormal cytokine expression.[40] Consistent with the latter view, cytokine modulation rather than direct cytotoxicity underlies the predominant mechanism of action for some of the currently available JAK inhibitors, which have recently been shown to have palliative value in myelofibrosis.[41,42] JAK–STAT is not the only abnormal pathway in MPN and it might not even be the most important for clonal evolution.[18] Effective abrogation of clonal myeloproliferation and leukemic transformation in MPN might require therapeutic targeting of other pathways, in addition to or instead of JAK–STAT.

Conflict of interest

The author declares no conflict of interest.

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