Epigenetics and mutations in chronic myeloproliferative neoplasms

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The BCR-ABL1-negative classic myeloproliferative neoplasms, polycythemia vera (PV), essential thrombocytemia (ET) and primary myelofibrosis are clonal stem cell disorders associated with an increased production of mature blood cells belonging preferentially to one cell linage.[1] They share substantial phenotypic mimicry, can undergo phenotypic shifts (from PV to ET and vice versa) as well as evolution to myelofibrosis (post-PV/post-ET myelofibrosis), and all eventually progress to leukemia. The hypothesis that hypersensitivity of hematopoietic stem and progenitor cells to cytokines might largely account for the pathogenesis of myeloproliferative neoplasms has been corroborated by the discovery of mutations that affect cytoplasmic proteins involved in cytokine signaling, either resulting in a gain-of-function (JAK2 and MPL) or a loss-of-function (CBL and LNK). Dysregulation of tyrosine kinases is a recurrent theme in chronic myeloid neoplasms, as exemplified by the constitutive activation of ABL caused by oligomerization of the BCR-ABL fusion protein in chronic myelogenous leukemia, the gain-of-function mutation of the tyrosine kinase receptor c-KIT in mastocytosis, and the activation of platelet-derived growth factor receptor-α or -β and fibroblast growth factor receptor in hypereosinophilic disorders. However, high-throughput genomic analyses of myeloproliferative neoplasms have recently identified a second group of mutations that affect proteins involved in the epigenetic regulation of transcription, such as TET2, ASXL1 and EZH2.[2] These abnormalities can occur in association and/or with mutations targeting tyrosine kinases.

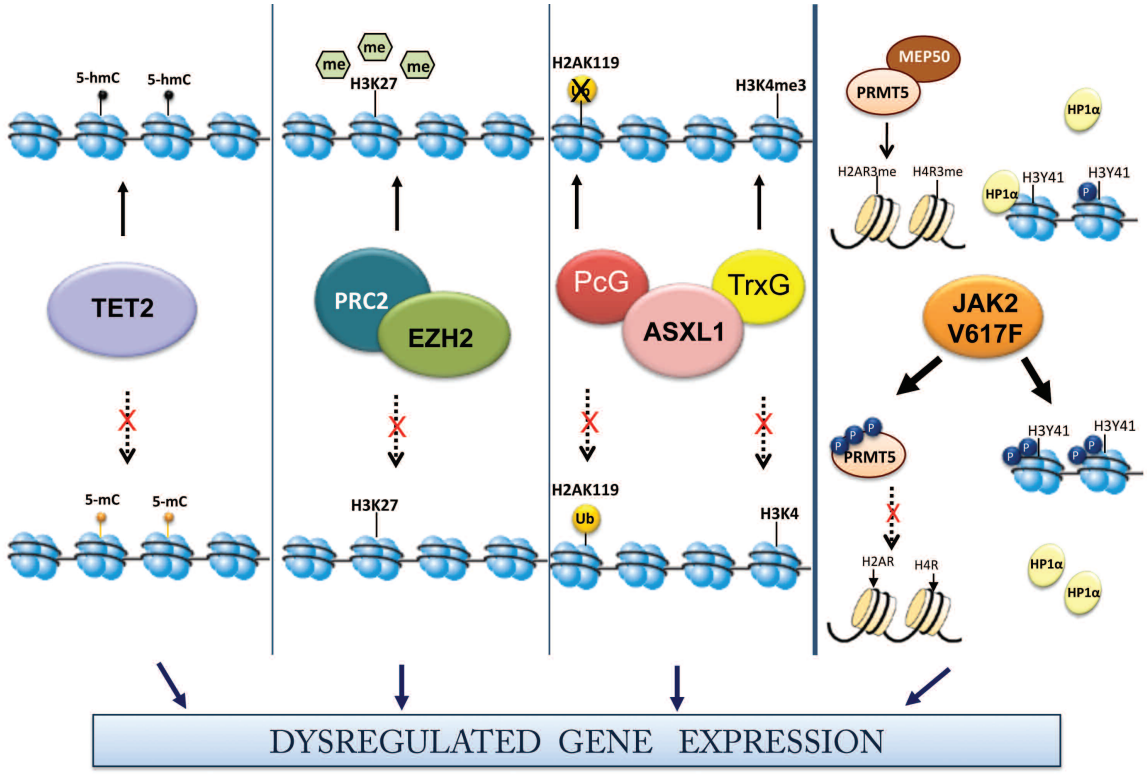

Figure 1. Known functions of EZH2, TET2, ASXL1 and JAK2 in epigenetic regulation of gene expression. The upper part of each box schematically depicts the main known function of each protein, while the effects of abnormal proteins are shown in the lower part. TET2 normally converts 5-methylcytosine (5mC) to 5-hydroxymethylcytosines (5hmC). EZH2 is the catalytic subunit of the PRC2 complex and trimethylates Lys-27 of histone H3 (H3K27) leading to transcriptional repression of target genes. ASXL1 exists in chromatin-associated multiprotein complexes, together with PcG and TrxG proteins, involved in modifications of chromatin configuration that result in repressed and enhanced transcription, respectively, in a cellular context-specific manner. Described loss-of-function mutations of EZH2, TET2 and ASXL1 presumably lead to suppression of catalytic activity of these enzymes. Mutant JAK2, but not the wild-type protein, phosphorylates protein arginine methyltransferase 5 (PRMT5), causing inhibition of its arginine methyltransferase activity on H2A and H4 (H2AR3me and H4R3me). JAK2 also phosphorylates Tyr 41 (Y41) on histone H3 leading to decreased HP1α binding to chromatin; the displacement of HP1α is magnified after enhanced H3Y41 phosphorylation due to JAK2V617F.

However, unlike the *JAK2V617F*, *JAK2* exon-12 and *MPLW515* mutations, which have been identified very rarely outside the classic myeloproliferative neoplasms, *TET2*, *ASXL1* and *EZH2* are mutated in a wide spectrum of myeloid malignancies including myelodysplastic syndromes, myelodysplastic syndromes/myeloproliferative neoplasms and acute myeloid leukemias, suggesting that these mutations might contribute a common genomic hit in myeloid malignancies. Abnormalities in other epigenetic regulators, due to mutations in IDH1 and IDH2 and DNMT3A, have been detected preferentially in association with leukemic transformation of chronic myeloproliferative neoplasms as well as in *de-novo* leukemias.

TET2, which stands for ten-eleven-translocation-2, is member of a family that includes also *TET1* and *TET3*. *TET2* is located on 4q24 and contains 11 exons. The founder of the *TET* family, *TET1*, was originally identified as a fusion partner of *MLL* in acute myeloid leukemia with the t(10;11)(q21;q32) translocation. One known function of TET proteins is to accomplish 5-methylcytosine hydroxylation resulting in the generation of 5-hydroxymethylcytosine (Figure 1); the significance and role of this modified base is still largely unknown, but 5-hydroxymethylcytosine has been found enriched in actively transcribed genes and in the promoters of polycomb-repressed elements that are normally activated during development of mouse embryonic stem cells.[3] Targeting *Tet2* in mice caused a progressive expansion of hematopoietic stem and progenitor cells leading to a myeloproliferative phenotype with splenomegaly, extramedullary hematopoiesis and marked expansion of the monocytic compartment.[4,5] *TET2* mutations have been discovered in a wide range of myeloid malignancies,[6] including classic myeloproliferative neoplasms (approximately 14%), mastocytosis, myelodysplastic syndromes, chronic myelomonocytic leukemia (CMML; 50%) and in post-myeloproliferative neoplasm or *de-novo* acute myeloid leukemia. Sequential analysis of the presence of *TET2* mutations during the progression of myeloproliferative neoplasms has shown that these mutations may precede or follow the *JAK2*V617F mutation[6,7] or occur at the time of disease transformation to acute myeloid leukemia.[8] Mutations are scattered over

Table 1. *ASXL1* mutations reported in the literature.

Reference	Disease type (N. of specimens)	*ASXL1* mutation frequency	Clinical correlation
Gelsi-Boyer V *et al.* Br J Haematol 2009; 145(6):788-800	MDS (n= 35) CMML (n= 44)	11% 43%	Not described
Carbuccia N *et al.* Leukemia 2009; 23:2183-6	PV (n=10) ET (n=35) PMF (n=10) MPN/AML* (n=5)	0 2.8% 30% 20%	Not described
Abdel-Wahab O *et al* Cancer Res 2010;70(2): 447-52.	MPN/AML(n=63)	19.3%	ASXL1 mutation detected both in chronic and blast phase of MPN
Pérez B *et al.* Br J Haenatol 2010;151:460-8	JMML (n=68)	4%	Not described
Rocquain J *et al.* BMC Cancer 2010;10:401	MDS (n=65) sAML (n=64)	18.5% 17.2%	ASXL1 mutations are associated with more aggressive forms of MDS
Szpurka H *et al.* Leuk Res 2010;34(8):969-73	RARS-T (n=20)	10%	Not described
Chou WC *et al.* Blood 2010; 116(20):4086-94	AML (n=501)	10.8%	ASXL1 mutations associated with older age, male sex, isolated trisomy 8, RUNX1 mutation; were inversely associated with t(15;17), complex karyotype, FLT3–internal tandem duplication, NPM1 or WT1 mutations; were also association with a shorter OS
Abdel-Wahab O *et al.* Leukemia 2011; doi:10.1038/leu.2011.58	PMF (n= 46) post-PV/ET MF(n= 22), MPN/AML (n=11) CMML (n=15)	13% 23% 18% 20%	No association with outcome.
Thol F *et al.* J Clin Oncol 2011 ; 29(18):2499-506	MDS (N=193)	20.7%	ASXL1 mutations associated with an intermediate-risk karyotype.; shorter OS and LFS
Bejar R *et al.* N Engl J Med 2011;364(26):2496-506	MDS (n= 439)	14.4%	ASXL1 mutations were predictors of poor OS
Stein BL *et al.* Haematologica 2011; (this issue)	ET (n=41) PMF (n= 77) PV (n= 42) MPN/AML (n=6)	0 36% 2% 33%	ASXL1 mutations associated with anemia-directed therapy in MF patients

MDS: myelodysplastic syndrome; CMML: chronic myelomonocytic leukemia; PV: polycythemia vera; ET: essential thrombocythemia; PMF: primary myelofibrosis; MPN/AML: acute myeloid leukemia secondary to a preexisting myeloproliferative neoplasm (MPN); OS: overall survival; LFS: leukemia-free survival; sAML: secondary AML; JMML: juvenile myelomonocytic leukemia; RARS-T: refractory anemia with ringed sideroblasts with marked thrombocytosis.

the gene and consist of small insertions, deletions and nonsense mutations, all expected to result in a loss-of-function of the protein, and missense mutations affecting conserved amino acids in catalytically active regions. *TET2* alterations are most commonly heterozygous, suggesting that TET2 haploinsufficiency may be a mechanism sufficient for transformation, as indicated also by the phenotype of *Tet*$^{+/-}$ mice. Inhibition of TET2 catalytic activity is also driven by the neomorphic IDH1/2 mutant proteins.[9]

EZH2, located on 7q36.1, encodes for the PcG enhancer of zeste homolog 2, the catalytic component of the polycomb repressive complex 2 (PRC2) that methylates histone H3 at lysine 27 (H3K27me3). The SET domain of EZH2 (and EZH1) is specifically involved in the trimethylation of K27. H3K27me3 is a marker of inactive chromatin, as opposed to H3K4 trimethylation which is a marker of transcriptionally active status (Figure 1). EZH2 also associates with DNA-methyltransferases to direct DNA methylation. Macro- and micro-deletions of the genomic region containing EZH2 have been found in about 10% of myelodysplastic syndromes, with a few subjects presenting loss-of-heterozygosity due to acquired uniparental disomy.[10,11] Mutations of *EZH2* have been reported in patients with primary myelofibrosis, myelodysplastic syndromes, and myelodysplastic syndromes/myeloproliferative neoplasms;[10-12] they are scattered throughout the gene and include missense, nonsense and premature stop codons resulting in loss of function. Both monoallelic and biallelic mutations have been described. Contrariwise, an activating Tyr641missense mutation has been identified in lymphomas.[13] Thus, by controlling chromatin structure and gene accessibility, EZH2 may behave as a tumor suppressor or oncogene depending on the cellular context.

ASXL1 encodes the Additional SeX combs–Like protein-1 which is one of the three mammalian homologs of *Drosophila* Additional Sex Comb (*Asx*) gene, named after the fact that *Asx* deletion caused homeotic transformation due to dysregulation of *Hox* genes, whose spatially and quantitatively appropriate expression is essential for the

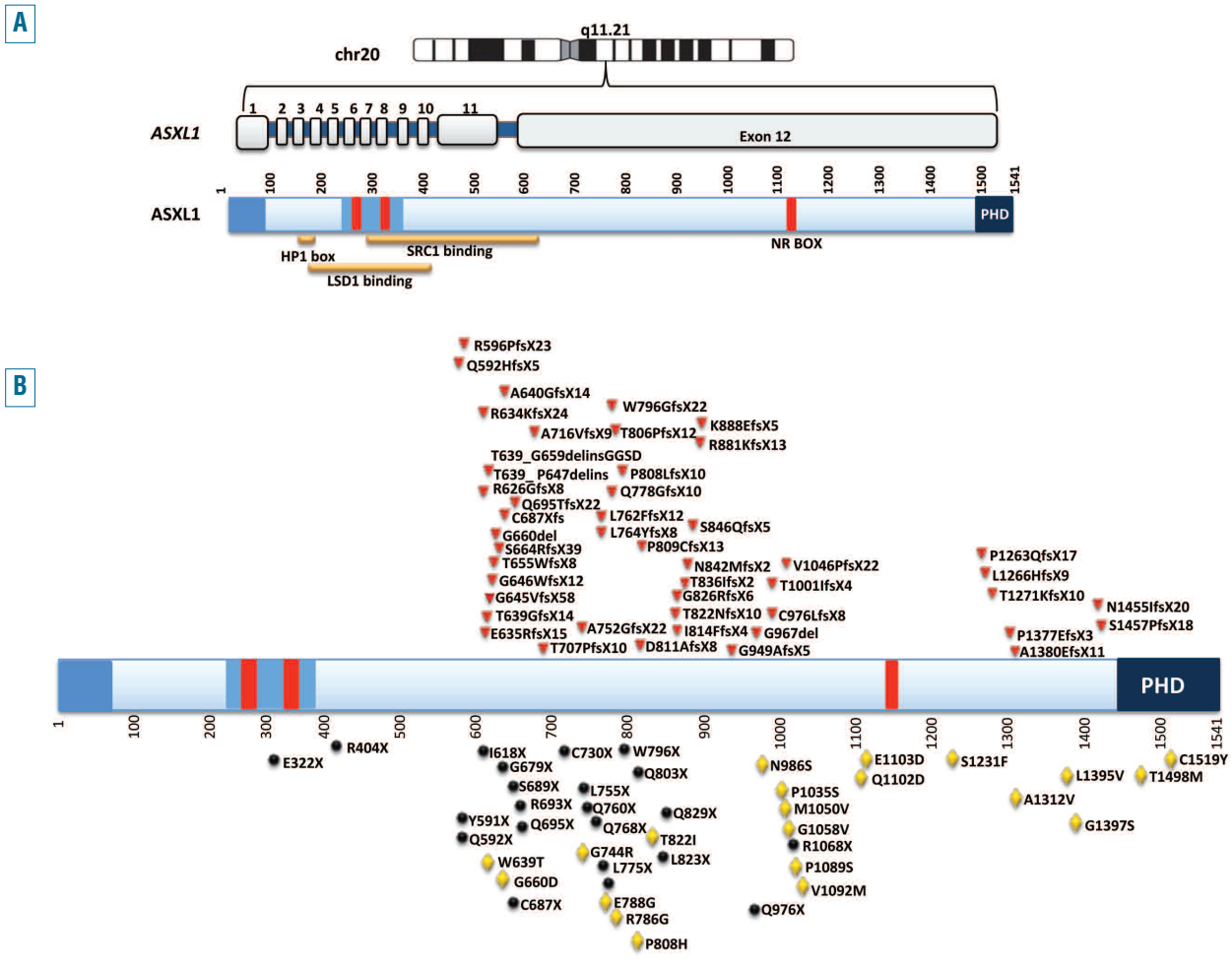

Figure 2. (A) Schematic representation of the human ASXL1 gene and protein. The conserved N-terminal ASX and the C-terminal plant homeodomains (PHD) are represented by light blue and blue boxes, repectively. Orange bars show HP1, LSD1 and SRC1 binding sites; red bars show nuclear receptor binding motifs (NR BOX). (B) Protein localization of described frameshift (red triangle), nonsense (black dot) and missense (yellow diamond) mutations of *ASXL1* (from publications listed in Table 1). Amino acid changes that were reported as unknown possible single nucleotide polymorphism are not depicted here.

anterior-posterior specification of axial structures during mammalian development. Constitutional *de novo* nonsense mutations of *ASXL1* have recently been described in half of the subjects with Bohring-Opitz syndrome (MIM605039), a disorder characterized by severe intellectual disability, distinctive facial features and multiple congenital malformations.[14] *ASXL1* maps to human chromosome 20q11.21, consists of 12 exons and encodes a protein composed of 1,541 amino acids (Figure 2A). All mammalian ASXL proteins have conserved sequence features: the amino-terminal ASX homology region, which contains two of the three putative nuclear receptor box domains, and a carboxy-terminal plant homeo domain finger (Figure 2A). It is a member of the enhancer of trithorax and polycomb (ETP) family that enlists proteins required for both the maintenance of activation and silencing of gene expression by modifying chromatin configuration. For example, ASXL1 can interact with retinoic acid receptor in the presence of retinoic acid and enhance the transcription of some genes while repressing that of others, depending on the cell context.[15]

The fine details of the mechanism of action of ASXL1 are not well defined yet, but the protein is involved in distinct multiprotein complexes that bind to and modify chromatin at target gene regions. Scheuermann *et al.* demonstrated that ASXL1 exists in a complex, named polycomb repressive deubiquitinase, with BAP1, a ubiquitin carboxy-terminal hydrolase that removes monoubiquitin from histone 2A in nucleosomes.[16] ASXL1 also associates with the histone acetyltransferase SRC-1, the histone methyltransferase MLL and forms a ternary complex with heterochromatin protein-1 (HP1) and the histone demethylase LSD. Thus, ASXL1 has pleiotropic and context-dependent repressive or activating effects on transcription through chemical modification of histones.

Frameshift mutations, nonsense mutations, and large 20q11 deletions of *ASXL1* have been described in 10-15% of myeloproliferative neoplasms and myelodysplastic syndromes, 40% of CMML (particularly in the myeloproliferative subset, 60%), in refractory anemia with ring sideroblasts and thrombocytosis, a few patients with chronic myelogenous leukemia and 15-20% of acute leukemias (Table 1). Most *ASXL1* mutations are found in exon 12, spanning the region from Tyr591 to Cys1519, and disrupt the protein downstream of the ASX homology domain with loss of the plant homo domain (Figure 2B). Germline targeted disruption of *Asxl1* in mice resulted in embryonic/perinatal death while in the few mice who survived to birth only mild hematopoietic defects were detected with no evidence of myelodysplastic or myeloproliferative disorder.[17] From a genetic point of view, the mutations in *Asxl1* deleted mice differ from the mutations seen in patients, which usually result in the deletion of the plant homeo domain finger while sparing its N-terminal motifs; this would suggest that *ASXL1* mutations generate a dominant-negative protein that can inhibit its wild-type counterpart.

In some studies, *ASXL1* mutation was associated with an unfavorable outcome in acute myeloid leukemia, CMML and myelodysplastic syndromes, while there is not enough information on this aspect in classic myeloproliferative neoplasms because of the relatively small series of patients (Table 1). In this issue of the Journal, Stein *et al.* report on 166 patients with myeloproliferative neoplasms who were analyzed for exon 12 *ASXL1* mutations.[18] Extending previous results, they detected *ASXL1* mutations very rarely in PV and ET, while the frequency reported in patients with myelofibrosis (included patients with primary and post-PV/post-ET myelofibrosis) was significantly higher (36%). Phenotypic correlations revealed a higher prevalence of anemia-directed therapy in *ASXL1*-mutated patients; however, the relatively low number of cases (n=77) examined hampered analysis of prognostic correlations.

Several lines of evidence indicate that mutations in tyrosine kinase (such as JAK2V617F and similar) are not sufficient for disease initiation and progression; rather, the JAK2V617F mutation can provide a proliferative advantage to progenitors during differentiation, allowing clonal dominance in late stages of differentiation, but it does not appear to target the stem cell.[19] On the other hand, it is becoming clearer that abnormalities of proteins involved in epigenetic regulation due to mutations contributed to the pathogenesis of classic myeloproliferative neoplasms and related myeloid malignancies, possibly targeting pivotal mechanisms affecting stem cell fate.[20] Furthermore, recent data suggest that there is a link between mutated tyrosine kinases and epigenetic regulators, since JAK2V617F has been shown to displace the repressor heterochromatic protein HP1α from chromatin by phosphorylating histone H3 at Tyr41,[21] and affect the methylosome by disrupting the association between protein arginine methyltransferase 5 (PRMT5) and its cofactor MEP50 following uncontrolled PRMT5 phosphorylation[22] (Figure 1).

Yet, there are many more questions left than answers provided. What is the hierarchy of mutations of epigenetic and tyrosine kinase genes in the events that lead to cellular transformation in myeloproliferative neoplasms? Indeed, does a defined hierarchy exist, or do mutations occur randomly? To which mutation, or group of mutated genes with overlapping functions, is the myeloproliferative neoplasm stem cell addicted? Is epigenetics a target for therapy in addition to/better than tyrosine kinase inhibition? Can known mutations predict response to treatment? These are complex questions that will certainly require even more complex experimental models. Perhaps, one piece of information that can be gained more rapidly from carefully performed clinical studies is whether any of these molecular abnormalities, singly or in combination, contribute to the phenotypic variability of myeloproliferative neoplasms and influence their prognosis.

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Chronic lymphocytic leukemia – genomics lead the way

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As the most common leukemia in the Western world, chronic lymphocytic leukemia (CLL) represents a prime example to demonstrate how modern genomic technologies have rapidly contributed to an improved understanding of cancer in general and how genomics have already started to translate into clinical practice.

In all cancers, characterizing the impact of the genetic aberrations on the phenotype of the disease has led to novel insights into the pathology underlying the disease. CLL is a neoplasm of mature B cells, a tissue in which the immunoglobulin genes are genetically modified to produce a functional B-cell receptor. The specific configuration of the immunoglobulin genes therefore gave further insight into the differentiation process of the malignant clone.[1] While non-malignant B cells use the full repertoire of immunoglobulin gene segments to produce the largest possible number of different B-cell receptors, in CLL cells the usage of immunoglobulin heavy chain variable gene segments (*IGHV*) is drastically restricted and biased towards a limited number of genes.[2] In addition, the process of somatic hypermutation of the variable region, which in non-malignant B cells leads to a change in binding affinity, allows CLL patients to be stratified into two biological and prognostic subgroups according to whether they harbor mutated or non-mutated *IGHV* genes.[1] These genetic findings led to the current model of the pathomechanism of CLL, according to which stimulation by (self-) antigens provides a pro-survival and possibly pro-proliferative advantage for CLL (precursor) cells, most likely leading initially to oligoclonal and subsequently monoclonal selection of malignant cells (Figure 1B). This model was recently improved significantly by findings from a murine xenotransplant model. Here, hematopoietic stem cells