

Comment on Yang et al, page 328

# ASXL1 mutations gain a function

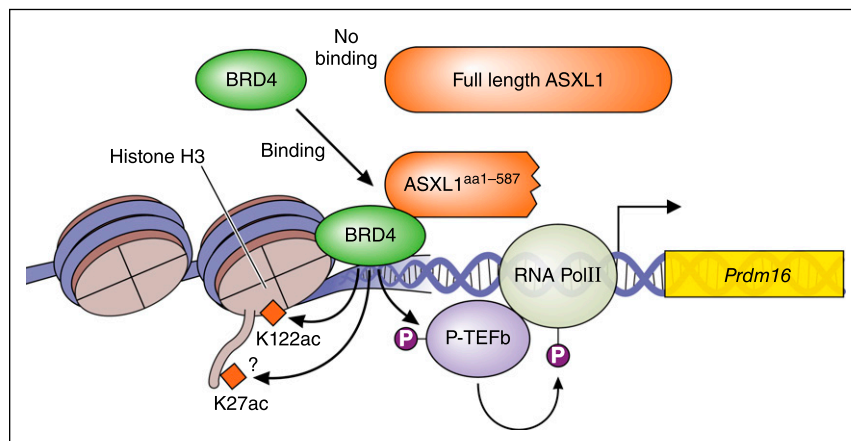
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**Whether additional Sex Combs-Like 1 (ASXL1) mutations are loss-of-function, dominant-negative, or gain-of-function mutations remains a point of considerable controversy. In this issue of *Blood*, Yang et al present clear evidence of a new “gain-of-function” role for an ASXL1 mutation.<sup>1</sup>**

Mutations of ASXL1 have been identified in a variety of myeloid neoplasms, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN), chronic myelomonocytic leukemia or MDS-MPN, and chronic myeloid leukemia, and are uniformly associated with poor prognosis.<sup>2</sup> It has been reported that knockdown or deletion of ASXL1 induces MDS-like symptoms, suggesting loss-of-function effects of ASXL1 mutations.<sup>3-5</sup> However, most ASXL1 mutants that have been identified in myeloid malignancies harbor nonsense or frameshift mutations localized near the 5' end of the last exon, which should thus escape nonsense-mediated decay of messenger RNA and produce a protein truncated at the C terminus. In addition, these mutations are always heterozygous, leaving 1 wild-type (WT) allele intact, implying either a dominant-negative or gain-of-function character.<sup>2</sup>

Inoue et al reported that the C-terminal truncating ASXL1 mutants induced an

MDS-like disease in a mouse bone marrow transplant (BMT) model.<sup>6</sup> They further showed that this C-terminal truncated ASXL1 protein was indeed expressed in MDS/AML cell lines harboring ASXL1 mutations.<sup>7</sup> In light of these findings and other findings, it is now generally accepted that the ASXL1 mutations are dominant-negative or gain-of-function mutations. However, it remains unclear how the ASXL1 mutants produce dominant-negative effects, or what functions they gain through truncation. Dominant-negative effects of the truncating ASXL1 mutants are suggested based on the decreased methylation of histone H3K27 (H3K27me3) mediated by ASXL1/PRC2,<sup>7</sup> but solid evidence supporting this notion is lacking. With respect to gain-of-function features of ASXL1 mutations, Balasubramani et al have reported that the ASXL1 mutant enhances the activity of BAP1, leading to deubiquitination of histone H2AK119 (H2AK119Ub)<sup>8</sup>; however, the full-length ASXL1 also enhances the BAP1 activity.<sup>9</sup>



ASXL1 mutation is a gain-of-function mutation. The ASXL1 truncating mutant (ASXL1<sup>aa1-587</sup>) but not wt ASXL1 recruit BRD4. BRD4 acetylates H3K122 and activates pTEFb leading to phosphorylation (P; activation) of RNA pol II. This results in enhanced transcription. Professional illustration by Patrick Lane, ScEYence Studios.

In this issue, Yang et al present compelling new evidence that ASXL1<sup>aa1-587</sup> acts as a gain-of-function mutant; a truncated mutant ASXL1 (ASXL1<sup>aa1-587</sup>) recruits BET bromodomain-containing protein 4 (BRD4) to genes, leading to the enhanced expression of genes including *Fos* and *Prdm16* (see figure), something that full-length ASXL1 does not do. That is, Yang et al have shown that ASXL1<sup>aa1-587</sup> (but not WT ASXL1) binds BRD4 and activates transcription of genes via phosphorylation of RNA polymerase II, presumably mediated by activation of the pTEFb complex and acetylation of H3K27/H3K122, both mediated by BRD4 (see figure). Using assay for transposase-accessible chromatin with high throughput sequencing of cKit<sup>+</sup> cells derived from a transgenic (Tg) mouse with Vav-1 promoter-driven expression of ASXL1<sup>aa1-587</sup> in hematopoietic cells, they show that progenitors of the Tg mouse present a slightly relaxed chromatin conformation compared with that of normal progenitors. Consistent with this open chromatin status, there are more upregulated genes than downregulated genes in cKit<sup>+</sup> cells of the Tg mouse. The upregulated genes include mainly hematopoietic stem cell (HSC) and myeloid pathway-related genes. The authors focused on *Prdm16* and *Fos*, which play important roles in HSCs, confirmed the upregulation of these genes by real-time polymerase chain reaction, and demonstrated that H3K122Ac and binding of BRD4 around the *Prdm16* gene are increased in cKit<sup>+</sup> cells in the Tg mouse. Additional studies using chromatin immunoprecipitation sequencing analysis will be needed to clarify whether transcription of the most upregulated genes in the Tg mouse are controlled in a similar way, via BRD4 and RNA polymerase II.

Overexpression of the truncated ASXL1 mutant induced MDS<sup>6</sup> in a BMT model, whereas a locus knock-in (KI) mouse of a C-terminally truncated ASXL1 mutant showed no pathogenic effect at any stage.<sup>10</sup> The Tg mouse described by Yang et al in contrast developed a variety of myeloid malignancies, including AML, MPN, MDS, and MDS/MPN. This discrepancy is likely due to the differences in the expression levels of the ASXL1 mutants in each model (that of the Tg model is approximately twofold that of the WT alleles), the length of each mutant (aa1-587 in the Tg model,<sup>1</sup> aa1-643 in the BMT model,<sup>6</sup> and aa1-643 plus 12 aa coded by the frameshifted sequence in the KI mouse model<sup>10</sup>), or the

distinct expression patterns regulated by the promoters (respectively, Vav promoter; long terminal repeat of retrovirus vector; and the authentic ASXL1 promoter). Another significant difference in the phenotypes of the Tg and locus Kl mice is that the numbers of HSCs and progenitors are increased in the former but not in the latter, as are the common myeloid progenitor/granulocyte-macrophage progenitor. Chimerism in competitive transplantation and the number of in vitro colony-forming unit cells are increased in the Tg model, but decreased in the locus Kl mouse. Thus, as a model for clonal hematopoiesis or preleukemia, the locus Kl mouse seems to be most appropriate. That notwithstanding, Yang's work deserves recognition for showing the first evidence for gain-of-function features of the ASXL1 mutations, which are frequently observed in myeloid malignancies and are always associated with poor prognosis. In addition, the present finding that BRD4 plays important roles in ASXL1 mutant-induced pathogenesis is clinically important and may pave a way to establishing a new strategy in treating the ASXL1 mutant-related hematological malignancies, including usage of HDAC inhibitors and the BRD4-bromodomain inhibitor JQ1, which inhibits BRD4 binding to chromatin.

**Conflict-of-interest disclosure:** The author declares no competing financial interests. ■

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## VASCULAR BIOLOGY

Comment on Ge et al, page 353

# Prochemerin processing by factor XIa

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**In this issue of *Blood*, Ge et al demonstrate a novel link between coagulation and inflammation by showing that factor XIa (FXIa) can generate the active chemoattractant and adipokine chemerin from its inactive precursor prochemerin.<sup>1</sup>**

The processes of innate immunity and hemostasis are intimately intertwined.<sup>2</sup> From an evolutionary perspective, and still seen in the horseshoe crab, both mechanisms originate from a single method to restrict and fight infections after injury to the organism. By capturing the pathogen in a clot, the intruder will be contained, and bleeding will be minimal. There is a lot of cross-talk between inflammation and hemostasis; coagulation markedly affects the inflammatory response, and proinflammatory cytokines and chemokines initiate and propagate coagulation activity.

Ge et al focus on the chemoattractant and adipokine chemerin (retinoic acid receptor responder gene 2). Chemerin is a proinflammatory molecule mainly expressed by white adipose tissue and the liver. Chemerin acts on a variety of cells (see figure) and is implicated in the inflammatory response, for instance, in psoriasis, metabolic syndrome, and cardiovascular disease.<sup>3</sup> Chemerin circulates as an inactive precursor prochemerin in plasma and needs to be converted by proteolysis into one of several active species. Biological activities of these species are regulated by further proteolytic cleavages to inactive chemerin forms. First, by screening a panel of enzymes from the coagulation, fibrinolytic, and inflammation cascades, Ge et al establish that FXIa specifically

generates a chemerin form that can be further processed into active chemerin by carboxypeptidases in the blood (CPN and activated thrombin-activatable fibrinolysis inhibitor). Strikingly, plasma kallikrein, which is highly homologous to FXIa, was very inefficient in processing prochemerin. The authors used a unique set of tools that specifically detected the different forms of chemerin, and they established that during activation of coagulation, prochemerin was processed, which coincided with the presence of FXIa. Processing of prochemerin in plasma was completely dependent on factor XI (FXI), because it did not occur in FXI-depleted plasma. The importance of FXI for prochemerin processing was confirmed in FXI-deficient patients, in whom the level of (intact) prochemerin was inversely associated with FXI levels in plasma. The action of FXIa was independent from its mode of activation, either by factor XIIa during contact activation or via feedback activation by thrombin. Taking all the data together, Ge et al's article demonstrates the importance of prochemerin processing by FXIa in plasma and provides a novel link between coagulation and inflammation.

FXI has been implicated before in inflammation. Recently, it was demonstrated that thrombin-mediated FXI activation on platelets amplified thrombin generation that promoted vascular inflammation, resulting