

## **INSIGHTS**

# JAK2/JAK2V617F heterodimers activate STAT1 and AhR to promote thrombocytosis

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In this issue of JEM, Zhou et al. (https://doi.org/10.1084/jem.20250153) report that hematopoietic progenitors heterozygous for the JAK2V617F mutation preferentially activate STAT1, promoting platelet production and thrombocythemia, whereas homozygous progenitors activate STAT5, leading to erythrocytosis and polycythemia.

The acquired gain-of-function mutation JAK2V617F can give rise to essential thrombocythemia (ET) in some patients, while in others, it leads to polycythemia vera (PV). The reason why the same mutation gives rise to different phenotypic manifestations of myeloproliferative neoplasms (MPNs) remains only partially understood. ET is characterized by increased platelet counts (thrombocytosis), while patients with PV display erythrocytosis with increased red cell mass together with variable degrees of thrombocytosis. A major step toward better understanding the basis of these genotypephenotype differences came from the discovery that blood cells in PV patients are frequently homozygous for the JAK2V617F mutation, whereas in ET patients, the mutation remains heterozygous (Scott et al., 2006). JAK2V617F can become homozygous through mitotic recombination on chromosome 9p, resulting in uniparental disomy of the telomeric region where the JAK2 gene is located (Kralovics et al., 2002). The strict association of PV with JAK2V617F homozygosity and ET with heterozygosity was later tempered by the detection of small proportions of homozygous progenitor cells in ET (Godfrey et al., 2012). The new question arising from these data is why the homozygous subclone fails to gain dominance in patients with ET.

Zhou et al. (2025) now examined signaling in primary hematopoietic cells from patients with ET that are heterozygous for *JAK2V617F* and cells from patients with

PV that are predominantly homozygous. The experiments were carried out in megakaryocyte-erythroid progenitors (MEPs), the developmental stage at which lineage commitment to erythropoiesis or megakaryopoiesis is regulated. JAK2 proteins associate with the cytoplasmic domains of the erythropoietin receptor (EPOR) that controls erythropoiesis and the thrombopoietin receptor (MPL), the main regulator of megakaryopoiesis. Binding of cognate ligands to EPOR and MPL induces dimerization of the associated JAK2 proteins and activates their tyrosine kinase activity. Hematopoietic cells heterozygous for JAK2V617F express roughly equal amounts of wild-type and mutant JAK2 mRNA, and presumably also JAK2 proteins. Thus, by random distribution, dimeric EPOR and MPL receptors are expected to associate with two wild-type JAK2 proteins in 25% of cases and with two mutant JAK2V617F proteins in another 25%, while the remaining 50% are expected to associate with one wild-type and one mutant JAK2 protein. To distinguish the wild-type and mutant JAK2 and to study the formation of dimers between them, the authors used epitope-tagged versions of JAK proteins and performed proximity ligation assay (PLA), which allow to visualize close vicinity of two proteins.

MEPs isolated from cord blood were co-transfected with expression vectors carrying HA-tagged wild-type JAK2 and V5tagged mutant JAK2V617F. PLA analysis in





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these cells showed predominantly heterodimers between wild-type and mutant JAK2, whereas PLA analysis of MEPs transfected solely with V5-tagged JAK2V617F showed as expected homodimerization of the mutated JAK2V617F. To exclude heterodimerization with other JAK family proteins, MEPs transfected with V5-tagged JAK2V617F together with either Myc-tagged JAK1 or Flagtagged Tyk2 were analyzed by PLA but showed no heterodimerization with JAK2V617F. Using PLA analysis, the authors also found association between JAK2V617F/ JAK2 heterodimers with STAT1, while JAK2V617F homodimers associated with STAT5. These results are interesting and suggest that cells heterozygous for JAK2V617F signal largely through wildtype/mutant JAK2 heterodimers. However, the expression levels of the epitope-tagged proteins by far exceeded the expression levels of the native JAK2 proteins and the receptor proteins to which JAK2 normally

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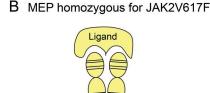
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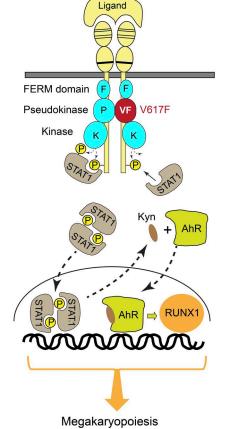
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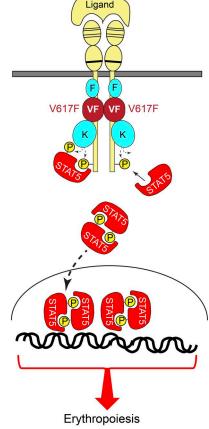




# A MEP heterozygous for JAK2V617F







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JAK2 signaling in MEPs. (A) Signaling in MEP heterozygous for JAK2V617F. Schematic drawing of a cytokine receptor (yellow) that is activated by ligand binding. JAK2 proteins are bound to the cytoplasmic domains of the dimeric cytokine receptor through their FERM domain (F) and preferentially form heterodimers between the wild-type JAK2 and the mutant JAK2V617F. The JAK2V617F mutation is located in the pseudokinase domain (VF), which dimerizes with the wild-type pseudokinase domain (P), thereby activating the kinase domains (K). The JAK2/JAK2V617F heterodimer preferentially phosphorylates STAT1, which then dimerizes and translocates into the nucleus where they activate transcriptional program that favors megakaryopoiesis. MEPs heterozygous for JAK2V617F express higher levels of Kyn. Kyn activates AhR to translocate into the nucleus, where it upregulates RUNX1 and activates transcription that biases MEPs toward megakaryopoiesis. (B) MEP homozygous for JAK2V617F form stable homodimers through their mutated pseudokinase domains and preferentially phosphorylates STAT5. STAT5 dimers translocate into the nucleus where they activate transcriptional program that favors erythropoiesis.

bind, and therefore may not faithfully reflect the in vivo conditions.

STAT proteins are major targets of JAK2 phosphorylation and serve as key mediators linking cytokine signaling to transcription. To assess STAT protein phosphorylation, MEPs were isolated from fresh bone marrow aspirates of patients with ET, patients with PV, and healthy controls, and analyzed by flow cytometry using antibodies specific for the phosphorylated forms of STAT1, STAT3, and STAT5. MEPs from ET patients showed increase in phosphorylated STAT1

(pSTAT1), but no changes in pSTAT3 or pSTAT5. In contrast, MEPs from PV patients showed increase in pSTAT5, but no changes in pSTAT1 or pSTAT3. These striking differences in STAT phosphorylation were observed without prior cytokine stimulation, suggesting that they reflect differential effects of JAK2V617F that depend on the presence or absence of wild-type JAK2. Signaling by JAK2 and JAK2V617F only occurs when bound to the cytoplasmic domains of receptors. MEPs are known to express both MPL and EPOR. The current report did not determine with which receptors the JAK2/JAK2V617F heterodimers associate in MEP cells. Activation of STAT5 by EPOR is known to be required for promoting erythropoiesis, whereas MPL can stimulate megakaryopoiesis by activating STAT5 or STAT1 (Sattler et al., 1995). However, megakaryopoiesis can also be stimulated by IFNy and its receptor that uses JAK2 and activates STAT1 (Huang et al., 2007). IFNy signaling activated by mutant JAK2V617F was previously shown to promote megakaryopoiesis in ET (Chen et al., 2010).

To establish a mechanistic link between

JAK2V617F heterozygosity, preferential STAT1 phosphorylation, and increased platelet production, the authors examined the activity of the transcription factor aryl hydrocarbon receptor (AhR), which they had previously shown to upregulate RUNX1 and thereby bias MEP differentiation toward the megakaryocytic lineage (Zhou et al., 2023). They found that the expression of RUNX1 and two AhR target genes (CYPIAI and CYPIBI) was elevated in MEPs from ET patients compared with those from healthy controls or PV patients. AhR is a cytoplasmic protein that becomes activated upon binding endogenous ligands and subsequently translocates to the nucleus, where it acts as a transcription factor. One such activating ligand, the tryptophan metabolite kynurenine (Kyn), was present at higher intracellular levels in MEPs of JAK2V617Fpositive ET patients compared with PV or healthy controls. Production of Kyn from tryptophan is catalyzed by the enzyme IDO1, which was upregulated at both the mRNA and protein levels in MEPs of ET patients. The IDO1 inhibitor 1-MT decreased Kyn levels in MEPs of ET patients and reduced AhR nuclear localization. 1-MT as well as the AhR antagonist SR1 also reduced platelet production in triple-immunodeficient NCG-X mice transplanted with CD34+ cells from ET patients, without altering red cell parameters. However, SR1 also promoted ex vivo expansion of human CD34+ cells (Boitano et al., 2010) and increased the capacity of peripheral blood CD34<sup>+</sup> cells to generate proplatelet-producing MKs and platelet-like elements (Strassel et al., 2016). Therefore, the prospect of targeting the IDO1-AhR axis to reduce thrombocytosis in ET patients will need to be validated by additional preclinical testing.

Since not only ET patients but also most PV patients display thrombocytosis, the main difference between ET and PV is the



involvement of erythropoiesis. The lack of STAT5 activation in MEPs from ET patients could explain the absence of erythrocytosis in ET. The data obtained in human MEP cells also differ from findings in mouse models of *JAK2V617F*-driven MPN. *Jak2V617F* knockin mice, despite being heterozygous, show PV phenotype and STAT5 activation (Hasan et al., 2013; Mullally et al., 2010). Genetic deletion of STAT1 in *JAK2V617F;Stat1*-/-double transgenic mice slightly reduced platelet counts and increased red cell parameters compared with *JAK2V617F;Stat1*-/+controls but failed to normalize platelet counts (Duek et al., 2014).

The structural basis of homodimeric JAK2 activation is quite well understood. Dimerization of the pseudokinase domains is essential for activation of the JAK2 kinase domains in both cytokine-stimulated wildtype JAK2 and mutant JAK2 proteins harboring pathogenic pseudokinase-domain mutations, such as V617F (Abraham et al., 2024; Glassman et al., 2022). The crystal structure of the JAK2V617F pseudokinase dimer and the cryo-EM structure of the homologous JAK1V657F mutant revealed structural differences relative to the wildtype JAK2 pseudokinase dimer. The JAK2V617F mutation induces an intermolecular π-stacking network, hydrogen

bonds, and salt bridges across the pseudo-kinase dimer interface, thereby stabilizing the JAK2V617F pseudokinase dimer and facilitating transactivation of the kinase domains. However, information on the structure of the JAK2/JAK2V617F hetero-dimer and its impact on the strength and specificity of downstream signaling is currently lacking. Such data could clarify why the JAK2/JAK2V617F heterodimer preferentially stimulates megakaryopoiesis but fails to activate erythropoiesis.

Overall, the report by Zhou et al. provides important new insights into the genotype-phenotype correlation and the molecular pathogenesis of ET and shows that selective STAT1 activation by the JAK2/JAK2V617F heterodimer plays a critical role in engaging AhR-driven transcriptional responses that promote megakaryocyte differentiation and increased platelet production.

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submitted work. O. Silvennoinen reported other from Ajax Therapeutics outside the submitted work; in addition, O. Silvennoinen had a patent to dual activity of kinase domains and uses thereof (US patent no. 8,841,078) issued.

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