Next, they performed microarray profiling of the co-cultured osteoblasts and found IL6, a proproliferative cytokine involved in bone metastasis (Ara et al., 2009), among the upregulated genes. Using a combination of strategies, the authors nicely demonstrate that IL6 is a downstream target of the Jagged1-Notch-Rbpj-Hey1 cascade, and is released from osteoblasts to promote tumor proliferation.

Notch signaling is a central pathway for regulating cell-cell interaction during embryonic development and is also involved in the pathogenesis of skeletal diseases (Tao et al., 2010). Hence, it is not unexpected that pharmacological inhibitors that block Notch pathway signaling will have a beneficial effect in both co-cultured Jagged1-expressing tumor cells and bone cells, as well as in the mouse model. However, a remaining clinically relevant question is whether these inhibitors can inhibit the outgrowth of bone metastasis in patients. Another important area that is critical for clinical translation would be the effect of Notch inhibition in other components of the skeletal system (Engin et. al., 2008), as well as elsewhere in the body.

In summary, the work presented by Sethi et al. (2011) identified a novel "seed and soil" crosstalk mediated by the TGFβ-Jagged1-Notch-IL6 signaling network that promotes the outgrowth of bone metastasis. The knowledge gained in this study contributes to our understanding of the pathogenesis of bone metastases and aids in finding therapy against it. In addition, it opens doors for many remaining unanswered questions. Does Jagged1 activate Notch signaling in other bone marrow-residing cells? How does Notch pathway interplay with other factors involved in bone metastasis? Will the use of Notch inhibitors together with inhibitors of IL6 or/and TGFβ be synergistic in halting bone metastasis?

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HiJAKing the Methylosome in Myeloproliferative Disorders

Radek C. Skoda[1,*] and Jürg Schwaller[1]
[1]Department of Biomedicine, University Hospital Basel, Hebelstrasse 20, 4031 Basel, Switzerland
*Correspondence: radek.skoda@unibas.ch
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***JAK2* gain-of-function mutations have been shown to cause myeloproliferative neoplasms. In this issue of *Cancer Cell*, Liu et al. (2011) demonstrate that these JAK2 mutants, but not wild-type JAK2, directly phosphorylate PRMT5 and inhibit its arginine methyltransferase activity, establishing a link between mutant JAK2 and histone arginine methylation.**

Janus kinase 2 (JAK2) is a ubiquitously expressed intracellular tyrosine kinase that associates with the cytoplasmic domains of hematopoietic cytokine receptors and becomes activated upon these receptors binding to their cognate ligands. Activating mutations in *JAK2* have been found in the majority of patients with myeloproliferative neoplasms (MPN), which represent clonal hematopoietic stem cell diseases characterized by increased proliferation of the erythroid, megakaryocytic, or myeloid lineages. The vast majority of patients with MPN (about 80%) carry mutations in *JAK2* codon 617 that exchanges a valine with a phenylalanine (*JAK2*-V617F) (Skoda, 2007). This mutated JAK2-V617F is an activated tyrosine kinase that renders hematopoietic cells hypersensitive for signals from upstream cytokine receptors (Epo, Tpo, and G-CSF) and phosphorylates (STAT3 and STAT5), as well as other downstream signaling proteins. In a minority of patients (less than 3% of MPNs), *JAK2* mutations have been found in codon 539 (e.g., *JAK2*-K539L) or in neighboring codons, leading to a variant of MPN with selectively increased numbers of erythroid cells. The mutant JAK2 proteins were shown to activate proliferation, inhibit apoptosis, and interfere with genome stability. However, all

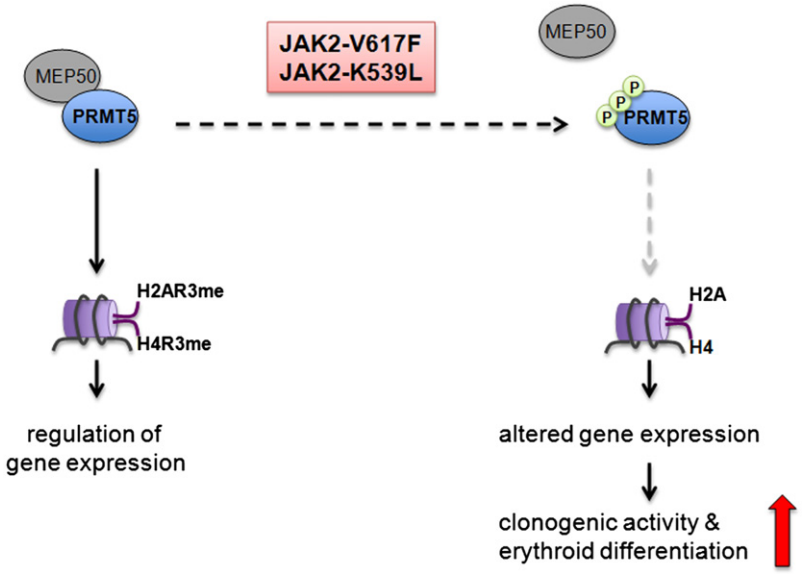

Figure 1. Oncogenic JAK2 (V617F, K539L) Phosphorylates PRMT5 and Disrupts the Methylosome

of these effects were also observed when the wild-type JAK2 was stimulated by cytokine receptor activation. The failure of SOCS3 to inhibit JAK2-V617F was so far the only hint that JAK2-V617F is not just a constitutively active form of wild-type JAK2 (Hookham et al., 2007). The work by Liu et al. (2011) describes phosphorylation of the protein arginine methyltransferase 5 (PRMT5) as a novel activity that is unique to the mutant JAK2-V617F and JAK2-K539L proteins.

Protein arginine methylation has emerged as an important mechanism to regulate protein-protein or protein-nucleic acid interactions. Today, nine human PRMTs are known that are classified into two major categories (class I and II), based on their substrate and reaction specificity (Krause et al., 2007). Class II PRMTs include PRMT5, PRMT7, and PRMT9, which catalyze the symmetrical dimethylation of arginine residues. PRMT5 activity was previously shown to be involved in epigenetic modulation of transcription through methylation of histones H2A, H3, and H4.

In yeast two-hybrid screenings, PRMT5 was previously identified as a JAK2 interacting protein. Liu et al. (2011) now found that mutant JAK2 (V617F, K539L) binds PRMT5 stronger than wild-type JAK2 and phosphorylates PRMT5 in vitro and in vivo, characterizing PRMT5 as a novel bona fide substrate of mutated JAK2 (Figure 1). Phosphorylation by mutant JAK2 of PRMT5 reduced its methyltransferase activity toward recombinant histones H4 and H2A. Liu et al. (2011) were also able to narrow down the residues in PRMT5 that are targeted by mutant JAK2 to Y297, Y304, and Y307. The N terminus of JAK2 upstream of the activating mutations interacts with PRMT5. Interestingly, phosphorylation of these PRMT5 residues seemed to disrupt the association of PRMT5 with MEP50, a cofactor for the enzymatic activity of the methylosome. shRNA-mediated knockdown of PRMT5 in human CD34+ cord blood cells increased colony formation in methylcellulose and erythroid differentiation in liquid cultures. In contrast, overexpression of the wild-type PRMT5 (but not an enzymatically inactive mutant of PRMT5) decreased hematopoietic colony formation. Although gene expression profiling provided a list of potential downstream effectors, the molecular mechanisms remain to be elucidated. Finally, increased phosphorylation of PRMT5 was detected in all ten MPN patients with mutant *JAK2* that were examined and interestingly also in one MPN patient with wild-type *JAK2*.

Liu et al. (2011) provide a novel link between oncogenic JAK2 activity and epigenetic gene regulation. Previous work implicating JAK2 in epigenetic events showed that activated JAK2 phosphorylates histone H3 at tyrosine 41 and thereby reduces the binding of the heterochromatin protein HP1a (Dawson et al., 2009). Exclusion of the repressor HP1a from chromatin results in the activation of genes that are not directly targets of STAT3 or STAT5. The amplification of a region of chromosome 9p24 in malignant lymphomas harbors, in addition to *JAK2*, another gene, *JMJD2C*, which demethylates histone H3 and thereby inhibits HP1a binding (Rui et al., 2010).

The work of Liu et al. (2011) raises many interesting questions and possibilities. First, previous studies have described a connection of the JAK/STAT signaling pathway with another PRMT family member, PRMT1. Initially, STAT1 was found to be methylated on arginine by PRMT1 to be protected from the inhibitory action by the PIAS1 protein (Mowen et al., 2001). Very recently, PRMT1 has been shown to directly methylate arginine 303 of PIAS1 and thereby increase the inhibitory activity of PIAS1 on STAT1-dependent transcription (Weber et al., 2009). It remains to be determined whether in analogy to PRMT1, PRMT5 might also methylate PIAS3 and interfere with STAT3-dependent transcription. Interestingly PRMT5 was recently found to physically interact with STAT3 and proposed to be actively involved in STAT3-mediated transcriptional repression (Tee et al., 2010).

Second, it will be important to address whether disrupting PRMT5 alone (or one of its cofactors) is sufficient to induce MPN. A conditional knockout of *PRMT5* in the hematopoietic system should provide the answer. Somatic mutations in *PRMT5* have so far not been described in MPN. Are the effects of decreased PRMT5 activity limited to decreased histone methylation or are other substrates, such as p53, involved?

Third, very recent work implicates increased PRMT5 activity in the tumorigenesis of mouse lymphoma and human esophageal carcinoma (Aggarwal et al., 2010). Here, MEP50 is phosphorylated by cyclin D1/CDK4 and leads to increased activity of PRMT5. This suggests that inhibitors of arginine methyltransferases could have therapeutic potential. In contrast, the results of Liu et al. (2011) imply that blocking PRMT5 would favor myeloproliferation. Currently, these

apparent opposing effects of PRMT5 activity on tumorigenesis need further investigation.

Fourth, the observation of increased phosphorylation of PRMT5 in CD34+ cells from one MPN patient without activating *JAK2* mutation suggests that other tyrosine kinases could phosphorylate PRMT5. Will impaired PRMT5 also be the consequence of uncontrolled activity of tyrosine kinases, such as mutated ABL, PDGFR, or FLT3, frequently found in chronic and acute malignant myeloproliferations?

Finally, since phosphorylation and inhibition of PRMT5 activity are properties specific for the mutated JAK2 proteins, inhibiting the interaction between mutant JAK2 and PRMT5 could potentially interfere with MPN pathogenesis without affecting the wild-type JAK2.

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Monoallelic Deletion of *NFKBIA* in Glioblastoma: When Less Is More

Amanda L. Rinkenbaugh[1] and Albert S. Baldwin[1,2,*]
[1]Lineberger Comprehensive Cancer Center, Department of Pathology and Laboratory Medicine
[2]Department of Biology
University of North Carolina, Chapel Hill, Chapel Hill, NC 27599, USA
*Correspondence: albert_baldwin@med.unc.edu
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Bredel et al. (2010) recently identified a subset of glioblastomas that harbor monoallelic loss of *NFKBIA*, which negatively affects patient prognosis. This finding raises new questions as to the role of IκBα and NF-κB in glioblastoma, the relationship between EGFR and NF-κB signaling, and potential therapeutic targets.

When we think of mutations that promote cancer, those used for textbook and review examples, the NF-κB pathway does not get a lot of attention. Yet NF-κB is clearly established as an important mediator of oncogenesis (Karin, 2006), and mutations in the immediate regulatory pathways leading to NF-κB activation have been characterized (Courtois and Gilmore, 2006). Importantly, well-established mutations that lead to cancer, such as activating mutations in Ras, function oncogenically through NF-κB activation (Bassères et al., 2010; Mayo et al., 1997; Meylan et al., 2009). New work from Bredel et al. (2010) demonstrates a fascinating monoallelic deletion of *NFKBIA*, in patient-derived glioblastoma multiforme (GBM). This gene encodes IκBα, a critical negative regulator of canonical NF-κB activation. The work suggests an interesting link between EGFR-induced signaling in GBM and the loss of IκBα.

NF-κB is a transcription factor comprised of homo- and heterodimers of five subunits: p65/RelA, RelB, c-Rel, p105/p50, and p100/p52. Under basal conditions, IκB molecules (IκBα, β, and ε isoforms) sequester p65- and c-Rel-containing dimers in the cytoplasm. Full-length p100 and p105 contain similar motifs to IκB and must be processed to yield active p50 and p52 subunits. Upon activation by cytokines or other stimuli, the IKK complex phosphorylates IκB, leading to its proteasomal degradation, which leaves NF-κB free to accumulate in the nucleus to control target gene expression. Genes regulated by NF-κB promote cell proliferation and survival, underlying the importance of this transcription factor both in normal cell responses and in oncogenesis (Karin, 2006).

Due to the integral involvement of NF-κB in promoting proliferation and the survival of cells of the hematopoietic system, it is not surprising that mutations in this pathway are observed in hematologic malignancies. c-Rel is often amplified in B cell malignancies and Hodgkin's