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Research Highlight

Janus Kinase 2: An Epigenetic 'Writer' that Activates Leukemogenic Genes

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Activation of Janus kinase 2 (JAK2) plays a critical role in normal hematopoiesis and leukemogenesis. Dawson et al. (2009; JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. Nature *461*, 819–822) report that JAK2 performs this function by displacing the heterochromatin protein HP1α from chromatin through phosphorylation of histone H3.

Activation of the Janus kinase 2 (JAK2) pathway plays an important role in both normal hematopoiesis and hematological malignancies (Neubauer et al., 1998; Tefferi et al., 2005). Past studies have demonstrated that the function of JAK2 in hematopoiesis and leukemogenesis is mediated through cytoplasmic phosphorylation of the various signal transducer and activator of transcription (STATs), which are subsequently translocated into the nucleus to activate STAT target genes (Schwaller et al., 2000). In a study recently published in *Nature*, Dawson et al. (2009) report that JAK2 can also activate the leukemogenic gene, *Imo2*, through phosphorylation of histone H3 in nuclei. This study reveals a novel mechanism by which JAK2 contributes to the leukemogenesis process.

JAK2 is a classic intracellular nonreceptor protein tyrosine kinase that associates with various receptors of cytokines, growth factors and hormones (Parganas et al., 1998). JAK2 is normally located in the cytoplasm and binds to a proline-rich region in the proximal membrane domain of various receptors. Upon association with their cytokines/ligands, the receptors undergo a conformational change which brings two JAK2 molecules in close proximity to phosphorylate each other. The activated form of JAK2 phosphorylates STAT proteins which translocate into the nucleus to up-regulate STAT target genes (Figure 1).

Previous studies have established a critical function for the JAK2–STAT signaling pathway in normal hematopoiesis and leukemogenesis. For example, a constitutively active form of JAK2 mutation (JAK2 V617F) has been identified in the majority of the patients with myeloproliferative disorders (MPDs), in more than 95% of the patients with polycythemia vera and ~50% of those with essential thrombocytosis and idiopathic myelofibrosis (reviewed in Ihle and Gilliland, 2007). The requirement of the JAK2–STAT axis, particularly JAK2–STAT5, in the development of MPDs is supported by mouse bone marrow transplantation experiments. For example, mice transplanted with bone marrow with enforced expression of JAK2 develop a myelo- and lymphoproliferative disease. JAK2-mediated bone marrow transformation requires the presence of STAT5 as mice reconstituted with STAT5a/b null bone marrow with enforced expression of JAK2 fail to develop the disease (Schwaller et al., 2000). Although STATs are clearly important for JAK2 downstream effectors, the studies by Kouzarides and colleagues demonstrate that JAK2 can also activate leukemogenic genes by phosphorylation of histone H3 (Dawson et al., 2009).

In this study, the authors first observed that both wild-type and the constitutively active mutant JAK2 (V617F) are located in both the cytoplasm and the nuclei of various human leukemic cell lines and primary CD34+ hematopoietic progenitors. They further demonstrated that JAK2 can phosphorylate histone H3 at tyrosine 41 (H3Y41) *in vitro*. Furthermore, using a site-specific antibody, they demonstrated that H3Y41 phosphorylation levels correlate with JAK2 activity *in vivo*. For example, H3Y41 phosphorylation levels are much higher in leukemic cell lines with constitutively active JAK2 compared with HL60 and γ2A cells which lack JAK2. In addition, cytokine-stimulated JAK2 activation increases H3Y41 phosphorylation in K562 and BaF3 cells. JAK2 appears to be the only kinase responsible for H3Y41 phosphorylation as treatment of cells with JAK2 inhibitors TG101209 and AT9283 suppresses H3Y41 phosphorylation both *in vitro* and *in vivo*. These data collectively establish JAK2 as an epigenetic 'writer' that phosphorylates histone H3 in nuclei.

To search for the molecules that 'read' and mediate the downstream biological consequence of this novel histone modification, the authors focused their attention on the heterochromatin protein 1 (HP1) as previous studies in *Drosophila* have demonstrated that activation of JAK2 leads to the disruption of HP1 binding to heterochromatin (Shi et al., 2006). Interestingly, they found that the affinity of HP1α to histone H3 depends on the phosphorylation status of H3Y41. *In vitro* peptide binding assays indicated that H3Y41 phosphorylation reduces the

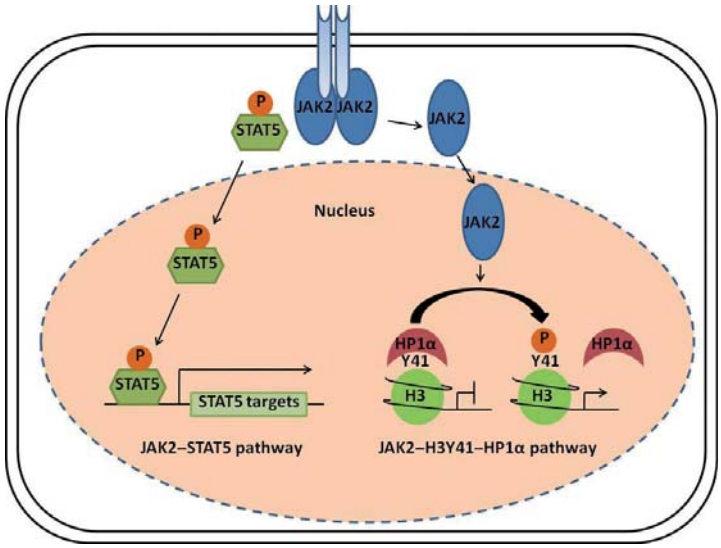

Figure 1 JAK2-mediated transcriptional activation involves two separate pathways. In the canonical JAK–STAT5 pathway, JAK2 is activated by autophosphorylation upon the association of receptors and their ligands. The active JAK2 further phosphorylates the STAT5, which is translocated into the nucleus to activate its target genes. Alternatively, the active JAK2 enters into the nucleus to phosphorylate histone H3 at tyrosine 41 (H3Y41). H3Y41 phosphorylation disrupts the association of HP1α with chromatin, which leads to the activation of oncogenes such as *Imo2*.

affinity of HP1α to histone H3 peptide. *In vivo*, only the unmodified form, but not H3Y41 phosphorylated histone H3 peptides, was able to displace HP1α from the heterochromatin speckles, which suggests that H3Y41 phosphorylation reduces the affinity of H3 to HP1α in a physiological context. Furthermore, inhibition of H3Y41 phosphorylation by treatment of JAK2 inhibitors increased the chromatin-bound HP1α in cells. By comparing gene expression between cells with and without treatment of JAK2 inhibitors, the authors found that some of the JAK2-regulated genes do not contain a predicted STAT5 binding site, indicating that these genes are regulated by signals other than JAK2–STAT5 pathway. Among these genes, *Imo2* is of particular interest because it is involved in both normal hematopoiesis and leukemogenesis (reviewed in McCormack and Rabbitts, 2004). Indeed, RT–PCR and chromatin immunoprecipitation assays demonstrated that treatment with JAK2 inhibitors causes the down-regulation of *Imo2* expression concomitant with decreased H3Y41 phosphorylation and increased HP1α binding at the *Imo2* transcriptional start site. On the basis of these results as well as the established function of HP1α in gene silencing, the authors concluded that constitutive activation of JAK2 can lead to oncogenic activation and genomic instability through direct phosphorylation of histone H3Y41 and displacement of HP1α from heterochromatin.

The results presented by Dawson et al. thus extend our understanding of the JAK2 regulatory network by revealing a novel JAK2-H3Y41-HP1α signaling pathway (Figure 1). It establishes an interesting molecular mechanism that links JAK2 enzymatic activity to histone phosphorylation and aberrant gene expression in leukemogenesis. In addition, the new JAK2 pathway provides a potential mechanism that links genome instability to hematopoietic malignancies (Plo et al., 2008). However, it is not clear whether these two JAK2 pathways are independent or can cooperate with each other in transcription regulation, since the lack of putative STAT5 binding site does not necessarily exclude the binding of STAT5 to these genes in cells. For this reason, it is necessary to examine both STAT5 and HP1α binding at these JAK2-regulated genes by ChIP analysis. Furthermore, the study also raises several questions that need to be addressed in the future. For example, previous studies have established that HP1α binds predominantly to H3K9 methyl-mark of the constitutive heterochromatin mainly functioning to maintain the stable heterochromatin domains (Bannister et al., 2001; Hall et al., 2002). It is not clear how important it is for HP1α to silence specific oncogenes in normal cells. This question can be addressed by comparing the direct targets of HP1α in normal and leukemic cells using the ChIP-Seq technique in combination with colony assays or bone marrow transplantation assays to evaluate the effect of HP1α knockdown. The second question that is worth addressing is the relationship between H3S10 phosphorylation and H3Y41 phosphorylation. Previous studies have shown that HP1α binds to the methyl-H3K9 through its chromo domain. During mitosis, H3S10 phosphorylation reduces the affinity of HP1α to H3K9 methylation and leads to release of HP1α from chromatin. The demonstration that H3Y41 phosphorylation can also disrupt binding of HP1α to chromatin raised the question whether the two phosphorylation events are linked and whether they disrupt a similar interaction. The answers to these questions will rely on future structure studies. Finally, it is important to dissect the JAK2–H3Y41–HP1α pathway and the canonical JAK2–STAT5 pathway in terms of the genes each pathway regulates and their relative contributions to leukemogenesis. Elucidation of this question will not only be important for understanding the molecular mechanism of JAK2-mediated leukemogenesis but also important in designing the right approach in the treatment of leukemias.

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