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Chronic growth factor receptor signaling and lineage inappropriate gene expression in AML

The polycomb connection

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Acute myeloid leukemia (AML) is a heterogeneous disease characterized by different types of chromosomal and genetic lesions. However, in most cases it has been shown that at least two mutations are required for complete leukemic transformation to occur. For many years it was thought AML development requires two distinct classes of mutations: class I mutations consist of translocation or point mutations in transcription factor genes, causing a block in differentiation at the myeloid progenitor stage, and class II mutations occur in genes encoding signaling molecules that provide a growth and/or survival advantage to these immature progenitors, leading to the accumulation of leukemic blasts in patients with AML. However, this distinction is now becoming blurred.

The alteration of gene expression by cellular signals is a well-established fact, and signaling processes are major drivers of cell fate decisions in development. Differentiating cells communicate via signaling molecules to coordinate the establishment of different gene expression programs. This can occur by either directly modifying the activity of transcription factors to activate (or repress) genes, or by direct signaling to chromatin and altering global histone modification. It is therefore not surprising that deregulated signaling in leukemia is more than a provider of survival advantage. However, only a few studies have determined the actual mechanism of deregulation of gene expression as a result of aberrant signaling. A good example for this notion are activating V617F mutations in the JAK2 kinase, which occur in patients with polycythemia vera and a large proportion of patients with thrombocythemia, which are conditions that develop into AML. JAK2 directly acts in the nucleus and phosphorylates histone H3 at tyrosine 41, thus preventing the binding of Heterochromatin protein 1 α (HP1α) to histone H3 and leading to a relief of gene repression. In turn, inhibition of JAK2 signaling downregulates the expression of the oncogene *LMO2* along with a decrease in H3Y41ph mark and an increased binding of HP1α protein.[1]

Our recent work that was published in the journal *Blood* uncovered an additional mechanism involved in the pathogenesis of AML that involves the abrogation of polycomb repression.[2] Polycomb complexes are important for the establishment of correct gene expression patterns during hematopoietic development and for stem cell renewal.[3] Our study used t(8;21) AML expressing the repressive fusion protein RUNX1/ETO as model to show that these cells respond to aberrant signaling with a deregulation of gene expression. One of the hallmarks of t(8;21) AML is the lineage inappropriate expression of B cell genes, such as *CD19* and *PAX5*.[4] The transcription factor PAX5 is a master regulator of B cell development, but has also been shown to repress the expression of myeloid specific genes and to block differentiation when overexpressed in myeloid cells. In normal myeloid precursors, *PAX5* is bound by polycomb and is not expressed. We show that in t(8;21) cells *PAX5* is not a direct target of RUNX1/ETO repressive activity, but that expression is dependent on de-regulated KIT, and potentially also FLT3 and RAS signaling. We demonstrate that aberrant signaling from a mutant KIT receptor via MAPKAPK3 (3pK) causes loss of polycomb complex association from *PAX5* chromatin, loss of PcG-associated repressive chromatin marks, acquisition of active chromatin marks and the association of an elongating RNA-Polymerase II with the *PAX5* promoter (**Fig. 1**).[2] Our paper outlines an important new mechanism mediating de-repression of polycomb targets in leukemia. Previously, MSK1 signaling and H3S28 phosphorylation have also been shown to be involved in polycomb target gene derepression.[5,6] RAS transformed cells and primary AML cells with FLT3 mutations have been reported to have constitutively activated MSK1 signaling and elevated levels of H3S28Ph and H3S10ph.[7] An important aspect of our study is the fact that upregulation of *PAX5* is correlated specifically with t(8;21) AML, although KIT or other receptor tyrosine kinase mutations are prevalent in other types of AML. Aberrant signaling in t(8;21) AML therefore cooperates with other factors to upregulate *PAX5* and other genes. It is likely that aberrant signaling also causes the deregulation of gene expression in other types of AML. Synergizing factors will be different for each type of AML, and understanding the individual disease mechanism will require the determination of common or unique deregulated polycomb targets in a genetic background of different signaling mutations. Another important notion is that the polycomb-mediated maintenance of the silenced state of a cell-specific regulator gene in certain cell types is crucial, since its aberrant upregulation may contribute to the differentiation block. It is thus

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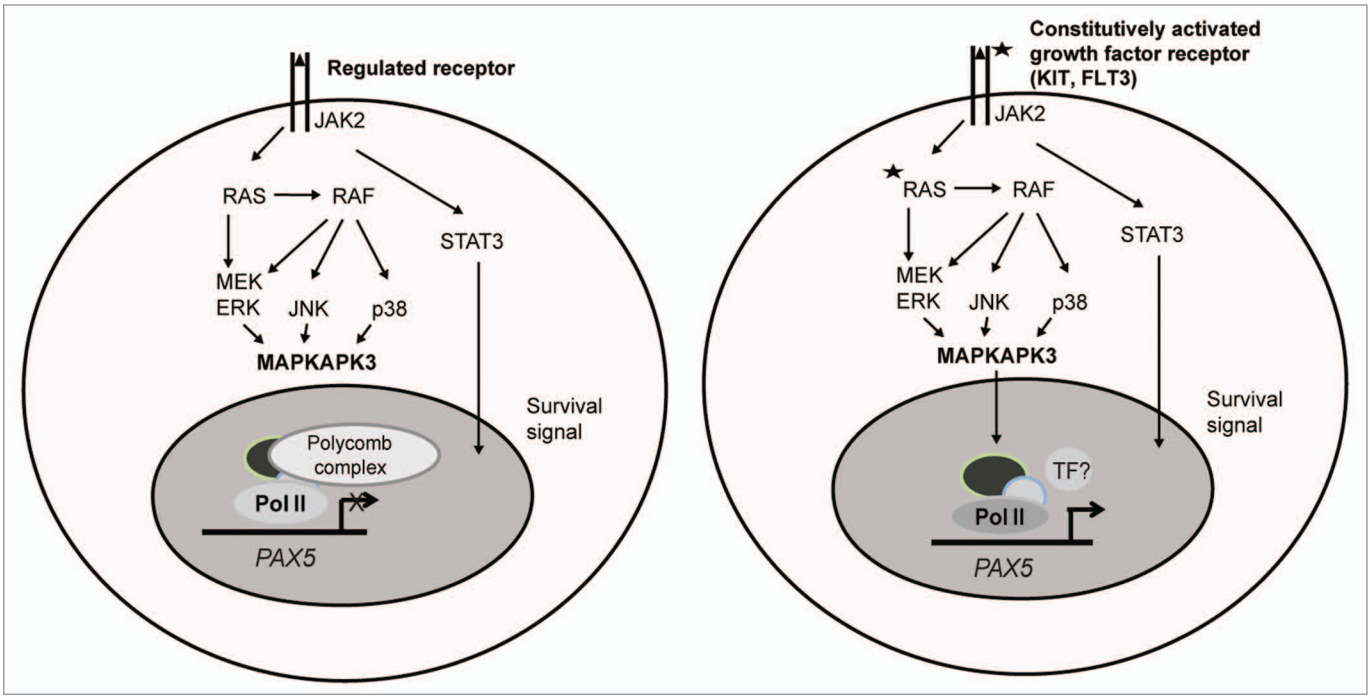

Figure 1. Constitutively activated growth factor receptors deregulate polycomb complexes via MAPKAP3 kinase, leading to the deregulation of *PAX5* gene in t(8;21) AML. Left panel: Receptor tyrosine kinases on binding to their respective ligands signal to the nucleus of the cell via JAK/STAT, MEK/JNK, and p38 pathways in a regulated manner. Right panel: In t(8;21) AML activating mutations constitutively activates receptor tyrosine kinases that, in turn, constitutively activates downstream kinases JAK/STAT, MEK/JNK, and p38. STAT3 provides survival a signal to t(8;21) cells. JNK, MEK, and p38 constitutively activate MAPKAPK3 (3pK), which leads to the dissociation of polycomb from *PAX5* leading its aberrant activation.

noteworthy that mutations in polycomb genes are recurrent features of AML.[8] To identify the precise mechanisms leading to the aberrant activation or repression of lineage-specific regulator genes will therefore be crucial for the understanding of the mechanism of oncogenesis.

Various signaling pathways have been targeted for therapeutic purposes in AML with only limited success, and the heterogeneity of mutations in AML poses a challenge against the development of a single drug. Studies dissecting the input of aberrantly activated signaling molecules on the deregulation of genes may, therefore, lead to the identification of novel, targetable shared signaling pathways.

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