Might as well face it: MLL’s addicted to HOX

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Overexpression of HOX.9 is a hallmark of MLL-rearranged leukemias. In this issue of Blood, Faber and colleagues show that HOX.9 is critical for proliferation and survival of leukemic cells.

Chromosomal rearrangements involving the MLL gene are a characteristic feature of a variety of human leukemias, including acute lymphoid (ALL) and acute myeloid leukemias (AML) as well as secondary leukemias resulting from treatment with topoisomerase inhibitors. The MEIS1 and HOX.9 genes have been shown to be major downstream targets of MLL fusion proteins. The mammalian HOX genes encode DNA-binding homeobox proteins that have a role in developmental patterns and tissue fate during embryogenesis. MEIS1 is a homeodomain protein that acts as a DNA-binding cofactor of HOX proteins from paralog groups 9 through 13. MEIS1 and HOX.4 genes are normally expressed in hematopoietic progenitor cells and their expression is down-regulated on differentiation. Overexpression of MEIS1 and HOX.9 is seen in a variety of leukemic cell lines and primary AML samples, suggesting roles for these genes in leukemia initiation and maintenance. In addition, fusion of HOX.9 to NUP98 in some AMLs further supports an important role for HOX.9 in leukemia.

In this issue of Blood, Faber et al ask if HOX.9 expression is required to maintain the leukemic state. The authors investigated the effects of knocking down HOX.9 expression in MLL-rearranged and nonrearranged human leukemia cells. After shRNA-mediated HOX.9 suppression, induction of apoptosis and decreased colony formation were observed in leukemia cell lines. An increase of cells in the G1 phase of the cell cycle and some cellular differentiation was also observed prior to the cells undergoing apoptosis. The apoptotic phenotype was rescued by expression of non-targetable HOX.9. The authors demonstrated that a greater induction of cell death was observed in MLL-rearranged cell lines and primary AML samples compared with MLL germ line cells after shRNA knockdown of HOX.9. In addition, the level of apoptosis positively correlated with levels of HOX.9 expression prior to shRNA knockdown. Gene expression profiling on MLL-rearranged cells after knockdown of HOX.9 expression showed decreased expression of genes, such as MEIS1 and PBX3, previously shown to play a role in leukemogenesis, suggesting these genes are downstream of HOX.9. Finally, MLL-rearranged cells were subjected to knockdown of HOX.9 and transplanted into mice. These mice showed a significant decrease in leukemia burden, demonstrating that continued HOX.9 expression is required for survival and proliferation of human MLL-rearranged cells in vivo.

The results provided by Faber et al demonstrate that MLL-rearranged cells are addicted to HOX.9 expression to maintain their leukemic state, as shown in the figure. Another major downstream target of MLL fusion proteins is MEIS1. Previous work done by Wong et al and work by Kumar et al used similar strategies to demonstrate that maintenance of the leukemic state in MLL-rearranged cells is also dependent on expression of MEIS1. Knockdown of MEIS1 expression results in decreased proliferation and survival of these cells. Although MEIS1 is important in maintenance of the leukemic state, elevated levels of MEIS1 are not sufficient to induce leukemia. In contrast, elevated levels of HOX.9 are sufficient to induce leukemia with a long latency. Coexpression of MEIS1 along with HOX.9, however, reduces the latency of HOX-induced leukemia. Taken together, these data suggest a role for both MEIS1 and HOX.9 in leukemia initiation and maintenance.

The data from the gene expression profiling experiment in Faber et al suggest that MEIS1 is downstream of HOX.9. Is continued HOX.9 expression required to maintain high levels of MEIS1 expression in leukemic cells? Knockdown of HOX.9 expression in MLL-rearranged cells and simultaneous ectopic expression of MEIS1 will further elucidate the complex interactions of these transcription factors in leukemia maintenance. Clarification of this gene partnership will lead to an understanding of gene regulation in MLL-rearranged leukemias. MEIS1 and HOX.9 have been shown to function as a heterodimer and in higher order complexes. Small molecule inhibitors could provide alternate therapeutic strategies by disrupting MEIS1/HOX.9 dimer formation, leading to loss of proliferation and survival in MLL-rearranged leukemic cells.

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REFERENCES
Platelet antigen-induced regulation in ITP

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The immune system has evolved several interactive peripheral regulatory mechanisms to protect against autoimmunity, and it has become increasingly clear that CD4+CD25+Foxp3+ T regulatory cells (Tregs) are an important component of this control.7 Their importance in maintaining peripheral tolerance is exemplified by the observations, for example, that mutation of FoxP3 leads to widespread autoimmunity in the scurfy mouse and immune dysregulation, culminating in the X-linked syndrome (IPEX) in humans.8 Treg deficiencies have been found in several autoimmune diseases including the autoimmune bleeding disorder, idiopathic thrombocytopenic purpura (ITP). It is currently thought that this deficiency is at the center of immune dysregulation and stimulation of autoimmune attack.1-6

Tregs can be generally divided into 2 flavors depending on their ontogeny and mode of action. For example, natural (n) Tregs are generated in the thymus and are generally anergic to antigenic stimulation but can effectively inhibit proliferation of CD4+ T helper cells via direct cell contact.7 Thus, the ability to isolate these nTregs holds the promise of immunosuppressive therapy, however, since they constitute only 3% of circulating CD4+ T cells, their numbers are far too small to be clinically effective. On the other hand, inducible (i) Tregs can be generated in the periphery from nonregulatory CD4+CD25− T cells and, although they share the phenotype and in vitro suppressive activities of nTreg, they are activated in an antigen-specific fashion. Nonetheless, although iTregs can be induced from CD4+ T cells, less is known about whether these cells function in healthy subjects or more importantly, whether they could be expanded from the peripheral blood of patients with autoimmune diseases known to be associated with deficiencies in Tregs.1-7 In this issue, Zhang and colleagues address the latter possibility by studying whether iTregs could be expanded from the peripheral blood of patients with autoimmune diseases known to be associated with deficiencies in Tregs.1-7 Thus, Zhang and colleagues address the latter possibility by studying whether iTregs could be stimulated in vitro from the peripheral blood of patients with ITP and whether the in vitro expanded cells were functional.8 What they found is fuel for the notion that ITP is not only a complex immunoregulatory disorder involving several cellular and soluble elements, but that perhaps an effective autologous cellular therapy for ITP could be developed.

The authors studied 41 newly diagnosed patients with chronic ITP. They found that platelet glycoprotein (GP)–specific induced iTregs could be generated de novo from nonregulatory CD4+CD25−CD45RA+ T cells and could mediate both antigen–specific and linked suppression of proliferating anti–platelet CD4+ T helper cells in vitro. Using a series of culturing techniques, they more importantly demonstrated that the expanded iTregs mediated their suppressive effects on T cells by actually modulating the T-cell stimulatory capacity of dendritic cells (DCs). This modulating effect was dependent on the presence of TGF-β, a potent immunosuppressive and tolerance-inducing cytokine. In an attempt to determine how the iTregs modulated DCs, they performed a genome-wide assessment of the DCs using microarrays and found that Toll-like receptor, Notch, and TGF-beta signaling pathways were related to the DC's ability to invoke suppression. How these particular signaling pathways within the iTreg-modified DCs affect the changes in their T-cell stimulatory capacity is still not known but these pathways are known to be intimately associated with both pro- and anti-inflammatory responses. It may be that the balance between these intracellular DC pathways ultimately controls the development of platelet autoimmunity. These findings not only increase our knowledge of how platelet-specific T-cell responses are regulated in patients with ITP but they shed light on the potential of producing antigen-specific iTregs from the patients in vitro for the purpose of antigen-targeted cellular immunotherapy.

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