Widespread over-expression of the non-clustered homeobox gene *HLX* in acute myeloid leukemia

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Homeobox (HOX) transcription factors are important for the regulation of normal hematopoiesis. Many cases of acute myeloid leukemia (AML) are driven by aberrant expression of clustered *HOX* genes that is mediated by chimeric oncoproteins involving MLL, chromosomal translocations directly affecting individual *HOX* genes, or perturbation of as yet unknown upstream regulators of *HOX* gene expression.[1] In contrast, much less is known about the role of non-clustered homeobox genes in leukemia. A recently published study has now established that overexpression of the non-clustered homeobox gene *HLX* is a common event in myeloid leukemogenesis and that this may have tangible clinical implications.[2]

Building on their previous finding that *Hlx* transcripts are increased in pre-leukemic hematopoietic stem and progenitor cells (HSPC) in a mouse model of AML,[3] Kawahara *et al.* observed that forced expression of HLX in murine HSPC resulted in depletion of long-term hematopoietic stem cells (LT-HSC) and enrichment of a more mature, granulocyte-monocyte progenitor (GMP)-like population with sustained clonogenic activity and a defect in terminal myelomonocytic differentiation. Interestingly, the loss of LT-HSC was not due to apoptosis or necrosis, suggesting that HLX may possess partial differentiation-inducing activity that leads to incomplete maturation of LT-HSC into GMP-like cells, a possibility that warrants further study.

In support of the idea that HLX promotes AML development, short hairpin RNA knockdown of HLX led to decreased viability, proliferation and clonogenicity of human AML cell lines and significantly prolonged survival in a murine AML transplantation model. Mechanistic studies showed that suppression of Hlx caused cell killing, mitotic arrest and myeloid differentiation. These cellular phenotypes were paralleled by induction of a distinct pattern of transcriptional changes, and modulation of individual genes from this signature, such as *PAK1* and *BTG1*, in cultured human AML cells partially mimicked the effects of HLX knockdown, suggesting that these genes are functionally relevant HLX downstream effectors.

In addition to these functional studies, Kawahara *et al.* also examined several publicly available microarray datasets and found that *HLX* mRNA expression is elevated compared to normal CD34-positive HSPC in nearly 90% of adult AML patients. Importantly, *HLX* overexpression was associated with inferior overall survival, independently of established prognostic parameters such as cytogenetic risk group or mutations in the *NPM1*, *FLT3* and *CEBPA* genes. These findings indicate that measuring *HLX* expression in AML patients may have immediate clinical value, a question that should be addressed prospectively taking into account the ever-expanding spectrum of molecular prognostic markers.[4] Furthermore, integration of the changes in gene expression induced by altering HLX levels in murine HSPC with the transcriptional profiles of human leukemia specimens with high or low *HLX* expression enabled delineation of a core set of HLX-dependent genes that can be viewed as a surrogate marker of HLX activity, and whose expression also correlates with survival in AML patients.

These compelling observations not only point to a fundamental role for HLX in myeloid leukemogenesis. Together with previous data demonstrating the leukemogenic activity of *CDX2*, which is also over-expressed in a substantial proportion of AML patients,[5,6] the data by Kawahara *et al.* suggest the intriguing possiblity that widespread misexpression of non-clustered homeobox genes represents a common mechanism of transformation that is shared by various AML subtypes. Finally, the findings by Kawahara *et al.* set the stage for future research. For example, the upstream events initiating aberrant *HLX* expression are still not known, and it will be interesting to further dissect how HLX deregulates its downstream effectors. Since targeting transcription factors is challenging and has not yet been realized in a clinical setting, understanding the entire molecular circuitry involving deregulated HLX expression may also help develop strategies to interfere with HLX activity for therapeutic benefit.

References

1. Argiropoulos B, Humphries RK. Hox genes in hematopoiesis and leukemogenesis. Oncogene. 2007;26(47):6766-76.
2. Kawahara M, Pandolfi A, Bartholdy B, Barreyro L, Will B, Roth M, et al. H2.0- like homeobox regulates early hematopoiesis and promotes acute myeloid leukemia. Cancer Cell. 2012;22(2):194-208.
3. Steidl U, Rosenbauer F, Verhaak RG, Gu X, Ebralidze A, Otu HH, et al. Essential role of Jun family transcription factors in PU.1 knockdown-induced leukemic stem cells. Nat Genet. 2006;38(11):1269-77.
4. Patel JP, Gönen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366(12):1079-89.
5. Scholl C, Bansal D, Döhner K, Eiwen K, Huntly BJ, Lee BH, et al. The homeobox gene CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis. J Clin Invest. 2007;117(4):1037-48.
6. Rawat VP, Thoene S, Naidu VM, Arseni N, Heilmeier B, Metzeler K, et al. Overexpression of CDX2 perturbs HOX gene expression in murine progenitors depending on its N-terminal domain and is closely correlated with deregulated HOX gene expression in human acute myeloid leukemia. Blood. 2008;111(1):309-19.