Insertional Mutagenesis Reveals Role of New Gene in Blood Cell Development

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A major safety concern of human gene therapy is the inadvertent development of cancer brought about by mutagenesis of chromosomal DNA, which halted a pivotal gene therapy clinical trial in the early 2000s. Retroviral-based vectors randomly insert genes in the chromosomes of target cells, which can lead to upregulation of proto-oncogenes or disruption of tumor suppressors. These side effects hinder the success of gene therapy with retroviruses; however, retroviral integration studies have proven to be a successful discovery tool in cancer research. Newer sequencing techniques allow researchers to elucidate where provirus integration takes place in the genome, providing a way to identify genes that influence cell survival, proliferation, and differentiation. In a study published in *Experimental Hematology*, researchers in the laboratory of Dr. Hans-Peter Kiem (Clinical Research Division) used retroviral integration analysis from stem cell gene therapy studies to identify and study a novel gene, *HOXC6*, in abnormal blood cell development.

The Kiem laboratory uses preclinical animal models to study hematopoietic stem cell (HSC) gene therapy. As an extension of this research, the investigators analyze the retroviral integration sites in HSC clones that repopulate the animals in the long-term after transplant. If the provirus inserts into a promoter or enhancer region, genes nearby are also upregulated and can influence HSC self-renewal. Through these studies, the researchers identified *HOXC6*, a member of the homeobox (*HOX*) gene family of transcription factors. While 22 of 39 individual *HOX* genes are expressed in mouse HSC, the role of many of these genes in hematopoietic differentiation is unknown. Wurm *et al*. studied the function of *HOXC6* by isolating and expressing the gene in mouse HSC with a retroviral-based vector. *HOXC6* overexpression enhanced the comparative repopulation of the bone marrow in a murine hematopoietic stem cell transplantation model threefold to fivefold. *HOXC6* overexpression also increased the formation of hematopoietic colonies in an *in vitro* assay. This effect was stronger than observed by overexpressing *HOXB4*, a *HOX* gene known to be involved in HSC self-renewal.

The researchers then examined how *HOXC6* overexpression specifically altered blood cell development. The percentage of T cells, B cells, and myeloid cells in the peripheral blood were

analyzed by flow cytometry 6, 12, and 16 weeks after transplant. *HOXC6* expression significantly biased differentiation towards the myeloid lineage ($p = 0.0013$) and decreased the number of B cells ($p = 0.0037$) compared to *HOXB4* expression. The researchers further confirmed that *HOXC6* overexpression inhibits B cell differentiation using an *in vitro* assay. In addition, *HOXC6*-overexpressing cells were retained in the bone marrow and significantly increased the numbers of early blood cell lineages known as common myeloid progenitors (CMP) and granulocyte macrophage progenitors (GMP). This suggests that *HOXC6* regulates self-renewal or induces long-term proliferation of hematopoietic progenitor cells. Importantly, the authors demonstrated that *HOXC6* is endogenously expressed in murine hematopoietic progenitor cells, strongly supporting a role for *HOXC6* in early lineage development.

In mouse models with *HOXC6* overexpression, 20% of the mice developed leukemia or a myeloproliferative disorder with increased immature myeloid cells. To determine the mechanisms of altered myeloid differentiation in *HOXC6*-expressing versus control cells, the researchers used gene expression analysis on sorted myeloid progenitor cells. *HOXC6* induced gene expression signatures consistent with stem/progenitor cells and similar to those found in acute myeloid leukemia (AML) patient cells. Furthermore, the researchers found the *HOXC6*-expressing retroviral vector integrated near a gene involved in myeloid malignancies, *Meis1*, which could contribute to leukemia formation. Notably, *HOXC6* expression is increased in human AML and acute lymphoid leukemia bone marrow.

According to Dr. Kiem "Through retroviral integration analysis from previous stem cell gene therapy studies, we identified *HOXC6* as a gene that could potentially expand blood stem cells. The study determined that *HOXC6* acts as a regulator in hematopoiesis and was implicated in malignant transformation. When overexpressed in a murine model it caused leukemia with a profile akin to AML." While normal levels of *HOXC6* maintain the self-renewal of stem cells, overexpression may promote leukemia formation by keeping cells in an immature stage. Taken together, this study shows the utility of using insertional mutagenesis to find novel genes involved in cancer development. As for gene therapy studies, improved viral vectors are being developed to more safely integrate genes without driving the expression of neighboring oncogenes.

Wurm, M, Kowalski, J, Heckl, D, Zhang, XB, Nelson, V, Beard, BC, Kiem, H-P. (2014) Ectopic expression of HOXC6 blocks myeloid differentiation and predisposes to malignant transformation. *Exp Hematol* 42: 114-125 e4.

See also: Baron BW1, Anastasi J, Hyjek EM, Bies J, Reddy PL, Dong J, Joseph L, Thirman MJ, Wroblewski K, Wolff L, Baron JM. 2012. PIM1 gene cooperates with human BCL6 gene to promote the development of lymphomas. *Proc Natl Acad Sci U S A* 10:5735-5739.

Kustikova OS1, Geiger H, Li Z, Brugman MH, Chambers SM, Shaw CA, Pike-Overzet K, de Ridder D, Staal FJ, von Keudell G, Cornils K, Nattamai KJ, Modlich U, Wagemaker G, Goodell MA, Fehse B, Baum C. 2007. Retroviral vector insertion sites associated with dominant hematopoietic clones mark "stemness" pathways. *Blood* 109:1897-1907.

Deneault E1, Cellot S, Faubert A, Laverdure JP, Fréchette M, Chagraoui J, Mayotte N, Sauvageau M, Ting SB, Sauvageau G. 2009. A functional screen to identify novel effectors of hematopoietic stem cell activity. *Cell* 137:369-379.

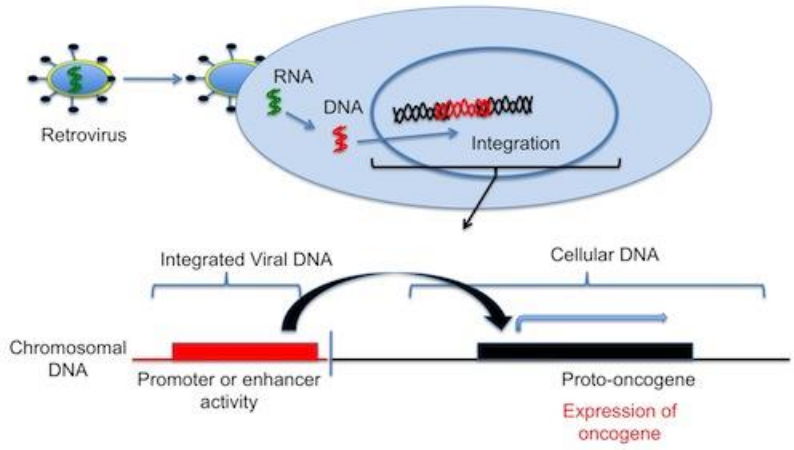

Image provided by John Kowalski

The integration of retroviral genes into host chromosomal DNA can occur at promoter or enhancer sites that drive the increase in proto-oncogene expression, leading to malignant transformation of the cell. Novel proto-oncogenes are identified through retroviral integration analysis. The function of these proto-oncogenes is determined by isolating and expressing the gene in an animal model to monitor gene expression changes and changes to cellular development.