a variant EMT phenotype induced by the tumor-specific antigen TM4SF5.

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4. Chang, Y.W., et al. 2005. CD13 (aminopeptidase N) tumor-specific antigen TM4SF5. Address correspondence to: Ruth J. Muschel, Radiation Oncology and Biology, The Radiobiology Research Institute, Churchill Hospital, University of Oxford, Oxford OX3 7JL, United Kingdom. Phone: 44-0-1865-857-427; Fax: 44-0-1865-857-533; E-mail: ruth.muschel@roh.ox.ac.uk.


The transcription factor homebox B4 (HOXB4) is a promising agent capable of providing a growth advantage to genetically modified hematopoietic stem and progenitor cells (HSPCs). In this issue of the JCI, Zhang and colleagues overexpressed HOXB4 in HSPCs from large animals using retroviral vectors (see the related article beginning on page 1502). Two years after transplantation, most animals developed leukemia, a consequence of combined HOXB4 and deregulated protooncogene expression. These results highlight the risks of combining integrating vectors and growth-promoting genes for clinical applications.

Hematopoietic stem cells and gene therapy

Hematopoietic stem and progenitor cells (HSPCs) are ideal targets for permanent genetic correction of defects in any lineage of hematopoietic cells. Most clinical applications have used gene transfer vectors based on integrating retroviruses, but the relative inefficiency of these vectors has limited the considerable potential of gene transfer into HSPCs. Genetically modified cells represent only a small fraction (1%–10%) of the hematopoietic cells after transplantation. Consequently, competition from unmodified infused and endogenous HSPCs may dilute any therapeutic effect of the transduced cells. This implies that this small fraction of genetically modified hematopoietic cells will correct diseases requiring more than 1%–10% of corrected cells only if they have a marked selective growth advantage in vivo.

Homeobox B4 promotes a selective growth advantage of transduced HSPCs

In most diseases considered to be suitable targets for gene therapy, corrected cells do not themselves have an inherent growth advantage, providing an impetus to arm retroviral vectors with genes capable of conferring a selective growth advantage to transduced HSPCs and their progeny in vivo. Ectopic expression of homebox B4 (HOXB4), a transcription factor containing a highly conserved DNA-binding motif known as the homeodomain, has been found to enhance HSPC self-renewal in vitro and in vivo and has been suggested as an
approach not only to enhance gene therapy efficacy, but also in other clinical settings where the numbers of HSPCs are limited, such as cord blood transplantation.

The impact of HOXB4 expression on HSPC self-renewal was discovered by Sauvageau et al., who demonstrated that transfection of normal mouse bone marrow cells with a retroviral vector overexpressing HOXB4 resulted in a dramatic increase in engraftment ability and HSPC number compared with marrow transfected with a control vector (1). Importantly, the recipient mice did not develop hematopoietic abnormalities or malignancies, suggesting HOXB4-expressing HSPCs were still subject to normal homeostatic controls (2, 3). Since these initial studies, it has additionally been reported that HOXB4 can expand primitive human progenitors in culture as well as those human cells able to engraft immunodeficient mice (4, 5).

**HOXB4 and leukemic transformation: the time has come**

Despite these encouraging findings, investigators have worried that inclusion of HOXB4 in gene transfer vectors could carry significant risks, particularly in the context of evolving concerns regarding vector-related insertional mutagenesis (6, 7). The strong enhancers contained in retroviral genomes can activate adjacent cellular genes following integration, but the risk posed by one or a few integrated vectors per cell was estimated to be very low. However, these assumptions were shattered in 2002, when French researchers announced that a child who had shown unequivocal clinical benefit in the HSPC gene therapy trial for X-linked SCID (SCID-X) (8, 9) developed a vector-related T cell acute leukemia three years after infusion of retrovirally modified CD34+ cells (10). Since then, three other children in the French trial and, more recently, one patient in a similar SCID-X study conducted by British researchers have developed leukemias. In at least three cases, the tumor cells had a clonal vector insertion activating the LIM domain only 2 (LMO2) transcription factor gene (11). It was hypothesized that cooperation between LMO2 and the growth-promoting effect of the corrective IL-2 receptor γ (IL2RG) transgene resulted in leukemic transformation, perhaps limiting the problem to SCID-X (12). However, 2 patients with chronic granulomatous disease treated in an HSPC gene therapy protocol have developed abnormal hematopoiesis and clonal expansion of vector-containing cells due to activation of protooncogenes (e.g., MDS-EVI7), despite the lack of growth-promoting activity of the corrective transgene (13). Our group also reported the development of vector-associated myeloid sarcoma in a rhesus macaque five years following transplantation of HSPCs transduced with a vector containing only a marker gene and a drug-resistance gene (14).

It has been demonstrated that high-level HOXB4 expression, achieved with retroviral (5) or adenoviral (15) vectors in human CD34+ cells, perturbed the myeloid differentiation program both in vitro (5, 15) an in vivo (5) without frank leukemia. Similarly, disturbed myeloid differentiation was observed in a multipotent hematopoietic cell line in vitro (16). Ectopic HOXB4 expression also proved to be key in the production of primitive mouse hematopoietic cells from embryonic stem cells then able to engraft irradiated recipients; however, these mice had abnormal hematopoiesis with enforced myeloid and suppressed lymphoid development (17). Despite these warning signs and continued concern regarding potential cooperativ-
ity between growth-altering transgenes and vector insertion sites, investigators continued to pursue the potential of the HOXB4 approach. In a study published in 2006, Kiem and his research group at the Fred Hutchinson Cancer Research Center reported preliminary results in their powerful non-human primate competitive repopulation model (14). They transduced monkey CD34+ cells with either a HOXB4-expressing vector or a control vector expressing only a marker gene and analyzed the competitive repopulating ability of these cells in vivo. As hoped, there was a very significant advantage for the HOXB4-transduced cells early following engraftment of the monkeys, but in contrast to mouse studies, a much less significant advantage for the HOXB4-transduced cells was observed long-term after transplantation (18).

In their study in this issue of the JCI, the same group now reports the first instances of leukemia linked to HOXB4 expression, both in the original group of monkeys now followed longer term and in dogs that received cells transduced with a HOXB4-expressing vector (19). Some 2 years after transplantation, 3 of 4 animals (2 of 2 dogs and 1 of 2 non-human primates) developed acute myeloid leukemias. Several lines of evidence strongly implicated HOXB4 in leukemogenesis. High-level HOXB4 expression was confirmed in the leukemic cells. Also, 3 of 4 animals followed long-term in this study developed leukemia, compared with none of more than 40 dogs and monkeys transplanted with cells transduced with similar vectors not expressing HOXB4 by the same research group and only 1 of our group (14). The most compelling evidence for the pivotal role of HOXB4 was provided by deriving a cell line from the leukemic cells of one animal and using short hairpin RNAs to knockdown HOXB4 expression cells of one animal and using short hairpin vectors not expressing HOXB4 and it remains unclear whether HOXB4 can expand human and primate long-term hematopoietic stem cells. Alternatively, HOXB4 may be transiently delivered to cells using nonintegrating retroviral vectors. “Suicide genes” such as herpes thymidine kinase could be included in vectors to provide the opportunity for delivering an exogenous “mission abort” signal via administration of ganciclovir if evidence for unchecked clonal growth appeared following gene therapy. However, killing efficiency is unlikely to be complete and, therefore, many problematic clones and subclones could persist after ganciclovir treatment.

Events in the recent history of gene therapy have led many to acknowledge that the field may have been, on several occasions, too eager to rush into the clinic. However, the wisdom gained from these setbacks is now becoming apparent in studies such as that of Zhang and colleagues in this issue of the JCI (19). Scientists and clinicians hoping to use their protocols to treat humans understand the importance of examining vectors’ behavior in a variety of comparative assays, including large animal studies, before entering the clinic. The use of well-characterized and well-tested preclinical models such as those of Zhang and colleagues is likely to minimize the risk of an adverse event that would throw the field into another crisis.

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