Gene therapy to manipulate the immune system?

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Researchers from the Johns Hopkins Kimmel Cancer Center (Baltimore, MD, USA) have introduced a marker gene into blood stem cells using a technique that enables the gene to be selectively activated in specific types of immune cell [1]. Although still in its early stages, this research is a step towards making it possible to use targeted gene therapy to boost or suppress an immune response. It could lead to future gene therapy techniques that help the body fight cancer or that prevent transplant patients rejecting their new organ.

Overcoming problems with gene transfer efficiency

Haematopoietic stem cells (HSCs) are produced by the body to replenish the entire haematopoietic system, including all the cells that contribute to immunity. It has long been a goal of gene technology to achieve high level transduction of a gene into an HSC and then to have that gene expressed in some, but not all, of the cell types that the parent cell gives rise to. However, this has proved difficult for several reasons, as Cynthia Dunbar at the Haematology Branch of the National Heart, Lung and Blood Institute (National Institutes of Health, Bethesda, MD, USA) points out. 'Early human trials showed that gene transfer success in murine models did not correlate with gene transfer efficiency in human stem cells; the levels of gene transfer were far below those required for therapeutic efficiency,' she says. Progress has been made to overcome this, both by improving transduction methodology using standard retroviral vectors, and by using safety-modified lentiviral vectors, such as HIV. 'Although,' Dunbar adds, 'extensive modifications of the HIV sequences in the vector genome have been necessary to reassure investigators that there is little possibility of recombination and productive HIV infection.'

The issue of selective expression

The other major hurdle, obtaining selective expression of a transgene in different cell types derived from the original transduced HSC, 'has been very difficult using standard retroviral vectors, which lose potency when strong lineage-specific control elements are included in their backbone', explains Dunbar. But progress is now being made, as the Johns Hopkins study by Linzhao Cheng and colleagues illustrates. 'Retroviral vectors based on murine oncoretrovirus and lentiviruses are currently the only way to achieve efficient and stable gene transfer to haematopoietic cells and many other primary cells,' explains Cheng. Building on previous research that made it possible to control the transgene using a chosen promoter rather than a viral promoter [2], Cheng's group constructed the EF–GFP vector, in which expression of the green fluorescent protein (GFP) gene is controlled by the promoter of a human housekeeping gene, EF1a. This promoter is constitutively expressed at high levels in all types of nucleated human cells. 'We also made the DR–GFP vector, in which expression of the GFP gene was controlled by the human HLA-DRa (MHC class II) promoter, a gene expressed only in antigen-presenting cells (APCs) and mature dendritic cells (DCs),' explains Cheng (Fig. 1).

Testing the new vectors

The new vectors were tested with human HSCs *in vitro*, and also *in vivo,* using the nonobese diabetic–severe combined immunodeficiency (NOD–SCID) mouse model. Both vectors efficiently transduced human pluripotent $CD34^+$ cells – cells that are capable of engrafting NOD–SCID mice – and the EF–GFP vector resulted in high-level expression of the GFP gene in all HSC progeny cells. By contrast, the DR–GFP vector led to gene transduction in all progeny cells but expression was limited. 'Transgene expression was found exclusively in human (MHC class II) HLA-DR^+ cells including differentiated dendritic cells; the cell types crucial for immune regulation,' says Cheng.

'This study demonstrates that lentiviral vectors can be used to achieve lineage-specific expression of a transgene in dendritic cells derived from transduced HSCs,' comments Dunbar. This, together with three other recent reports that lentiviral vectors can be used to achieve erythroid-specific high-level haemoglobin gene expression [3–5], has far-reaching implications, she points out. 'Specific expression of transgenes in dendritic cells could be important for tumour vaccine strategies. However, the results are more important globally as a demonstration that lentiviral vectors might be able to accommodate large, strong and complex genetic regulatory elements that can confer high-level expression and lineage-specificity. This could be crucial to the success of many experimental and therapeutic gene-transfer strategies,' stresses Dunbar (see also Ref. [6]).

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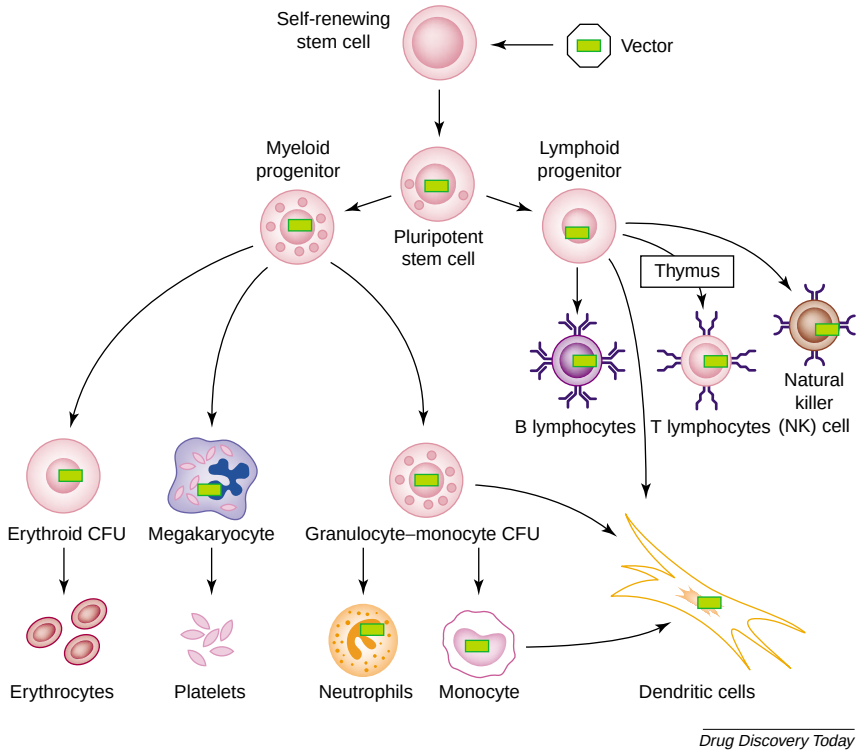

Figure 1. A vector delivers the 'targeted' or 'timed' gene (green bar) into haematopoietic stem cells, which form all the blood and immune cells but the gene remains silent. The green gene only lights up in developing dendritic cells (right corner), the most potent antigen-presenting cells. Figure kindly supplied by Linzhao Cheng (Johns Hopkins Kimmel Cancer Center, Baltimore, MD, USA).

Cheng's group has now embarked on a collaboration with Drew Pardoll, in the same department, to test the lentiviral vector in mice that have a tumour with a specific tumour antigen. 'Very potent, unprecedented immune responses were observed; we are currently monitoring long-term tumour-free survival of treated animals, as compared to sham-treated animals [controls] that die within 80 days,' reports Cheng. 'The trick will be to control the balance between immune activation and tolerance; nobody can yet be sure of the long-term outcome of these experiments,' he predicts. If it proves possible to break tolerance to cancer cells in tumour-bearing animals, this could lead to stronger cancer vaccines. The same approach could also be used to induce immune tolerance, which is also mediated by APCs and/or DCs. Another new project is looking at how the technique could be used to reduce rejection of embryonic stem cell transplants. 'By delivering a different set of genes, we may be able to reduce the immune responses either to self-antigens (in the case of autoimmune diseases) or to foreign antigens (in the case of donor transplants). Either way, we will learn more about the immune activation versus tolerance,' concludes Cheng.

References

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