Current Status of Gene Transfer into Haemopoietic Progenitor Cells: Application to Langerhans Cell Histiocytosis

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Summary A number of recent studies have shown that it is possible to obtain significant levels of gene transfer and expression in marrow progenitor cells and their progeny by using retroviral vectors. The data obtained from these studies and the possible applications to Langerhans cell histiocytosis (LCH) are reviewed.

Introduction

It is somewhat premature to discuss gene therapy for Langerhans cell histiocytosis (LCH) when so little is known of the molecular or cellular pathogenesis. However, the fundamental abnormalities will almost certainly reside in marrow derived cells, so that a brief review of the current status of gene transfer into haemopoietic progenitor cells may offer a useful background for future therapeutic strategies.

Although bone marrow progenitor cells are an appealing target for gene therapy, it has been difficult in large animal models to obtain long term or high level expression in haemopoietic progenitor cells and their progeny (Schuening et al., 1991; Van Beusechem et al., 1992; Lothrop et al., 1991). Because of the belief that human marrow progenitor cells would also be poorly susceptible to gene transfer/expression, therapeutic protocols using bone marrow cells were not originally considered feasible. In contrast, gene marking studies of human progenitor cells were considered justified, since these could potentially be used to determine the source of cancer relapses after autologous bone marrow transplantation even if the efficiency of gene transfer was low (Brenner et al., 1993a; Rill et al., 1992a; Rill et al., 1992b). In fact, these studies have proved to be informative not only about the source of relapse, but also about gene transfer and expression in normal progenitor cells (Brenner et al., 1993a,b). To date, all studies of marrow progenitor cells have used retroviral vectors, in part because they integrate in host cell DNA and are therefore present in all the progeny of the cell, but in part also because it is for these vectors that the most safety data are available. Other vectors are in development.

Gene marking of malignant cells in marrow

When patients with cancer relapse after autologous bone marrow transplantation, it may be because of residual disease in the patient or because residual disease is present in the infused marrow (Gale et al., 1989). This marrow is harvested before the intensive pre-transplant chemotherapy (intended to cure the patient) and so may contain malignant cells which return disease to an otherwise cured patient (Gorin et al., 1990; Shpall et al., 1991). If we were able to genetically mark residual malignant cells in the marrow at the time of harvest and detect these marked cells at the time of relapse, we would know directly that marrow contributed to malignant disease. The approach could then be adapted to study the efficacy of purging (Brenner et al., 1993a; Rill et al., 1992b).

Techniques for Gene Transfer

In order to obtain regulatory approval, the earliest marking protocols aimed at addressing this question used simple, though inefficient methods of gene transfer (Brenner et al., 1993a,b,c; Rill et al., 1991). Two × 10^6 marrow mononuclear cells were harvested from the posterior iliac crest of anesthetized children. Two-thirds of the marrow was immediately cryopreserved as a safety backup and one-third was exposed to either LNL6 or G1N retroviral vectors (supplied by Genetic Therapy Inc) (Bender et al., 1987; Miller et al., 1989) for 6 hours at a multiplicity of infection of p0.1. These vectors confer the neomycin resistance gene (Neoρ) which is used as a selectable marker. The cells were frozen and both the transduced and non-transduced marrow subsequently reinfused. Later clinical protocols have used growth factors during the time of transduction in attempts to increase efficiency of transfer (Bodine et al., 1989) and have also co-cultured marrow cells with vector producer lines to increase the number of rounds of infectious virus to which the target cells are exposed (Van Beusechem et al., 1992).

Marker Genes Show that Marrow Contributes to Relapse

We studied two malignancies of childhood; a haematologic malignancy, acute myeloblastic leukaemia, and a solid tumour, neuroblastoma, which may also invade the bone marrow. Two AML patients have relapsed. In both, the resurgent malignant cells contained the marker gene (Brenner et al., 1993b). Similarly, three neuroblastoma patients have relapsed, all with gene-marked neuroblastoma cells. The marker gene was detected in malignant cells in the marrow and in one patient at an extramedullary site, in the liver.

Thus marrow harvested in apparent clinical remission of both haematologic and non-haematologic malignancies may contain residual malignant cells which contribute to disease recurrence. We are now using gene marking to investigate the efficiency of purging.

Gene Transfer to Normal Cells

Of greater potential relevance to LCH, we found that the marker gene was transferred effectively to normal marrow progenitors. Approximately 10% of infused progenitor cells pre-transplant contained the marker gene and post-transplant this figure ranged from 0–15% in the 18 evaluable patients (Brenner et al., 1993b). The marker gene was present and expressed in progenitor cells of all lineages for up to 18 months post-transplant, the current duration of the study. The gene was also present and expressed in the mature progeny of these cells, including T cells, B cells and neutrophils. Thus, we believe that gene transfer into long-lived pluripotent progenitor cells in man is possible and that the transferred gene can be expressed long term, even in mature cells. We suggest the reason for the unexpectedly high efficiency of transfer and duration of expression is that the marrow we used was harvested from children after multiple rounds of intensive chemotherapy and therefore contained a
high proportion of early progenitor cells in cell cycle. It is likely that cells in cycle are more susceptible to retrovirus-mediated gene transfer (Bodine et al., 1991).

**Therapeutic studies**

Following on from these marker studies, a number of protocols in the USA and Europe have been approved for transfer of therapeutic genes to marrow progenitor cells. Studies of ADA deficiency and of Gaucher disease have opened, as have protocols to insert the multi-drug resistance gene into the marrow of patients with malignant disease. The aim of these last protocols is to increase the resistance of normal marrow to cytotoxic drugs and allow dose intensification without marrow ablation. There is no information as yet about the success of these approaches.

**Implications for LCH**

Even though we can obtain long term expression of transferred genes into a significant proportion of marrow-derived cells, therapeutic gene transfer protocols for LCH are not feasible until we know the appropriate cellular and molecular target. However, marker studies may be of value in patients receiving autologous bone marrow transplantation for severe and progressive LCH. Analysis of the patterns of marking cells in marrow, blood and lesions would give an accurate timetable of transfer for each cell type from the marrow to the lesions. Analysis of previous integration sites would provide information about the clonality of each cell type both within and between lesions. The integration information that would be gained by gene marking would likely contribute to our understanding of disease pathogenesis and would begin to pave the way towards definitive gene therapy for patients with severe multisystem disease.

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**References**


