Transfer and expression of the human multiple drug resistance gene as potential human gene therapy

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Abstract

The human multiple drug resistance (MDR) gene has been used as a model for human gene transfer which could lead to human gene therapy. MDR is a transmembrane protein which pumps a number of toxic substances out of cells including several drugs used in cancer chemotherapy. Normal bone marrow cells express low levels of MDR and are particularly sensitive to the toxic effects of these drugs. There are two general applications of MDR gene therapy: (1) to provide drug-resistance to the marrow of cancer patients receiving chemotherapy, and (2) as a selectable marker which when co-transferred with a non-selectable gene such as the human beta globin gene can be used to enrich the marrow for cells containing both genes. We demonstrate efficient transfer and expression of the human MDR gene in a retroviral vector into live mice and human marrow cells including CD34+ cells isolated from marrow and containing the bulk of human hematopoietic progenitors. MDR gene transduction corrects the sensitivity of CD34+ cells to taxol, an MDR drug substrate, and enriches the marrow for MDR-transduced cells. The MDR gene-containing retroviral supernatant used has been shown to be safe and free of replication-competent retrovirus. Because of the safety of the MDR retroviral supernatant, and efficient gene transfer into mouse and human marrow cells, a phase 1 clinical protocol for MDR gene transfer into cancer patients has been approved to evaluate MDR gene transfer and expression in human marrow.

Introduction

The safe and efficient transfer of human genes into target cells holds the promise of curing certain human diseases. This gene therapy may be accomplished either by providing the cells with a therapeutic normal gene to compensate for a missing or defective gene, or by adding a new gene with a new beneficial function to cells. The possibility of replacing defective genes in patients is being studied in diseases like cystic fibrosis and sickle cell disease. In each of these instances, the diseased organ can be reached by gene transfer: the pulmonary mucosa in cystic fibrosis, and the bone marrow in sickle cell disease. The disease can be potentially cured by adding the normal gene. We have chosen to study the human multiple drug resistance (MDR, MDR) gene and its transfer into bone marrow cells for its potential use in human gene therapy for several reasons: (1) bone marrow cells are easily accessible; (2) although we succeeded in transferring the neomycin-resistance gene into the bone marrow of live mice (Hesdorffer et al., 1990), and the human beta globin gene into mouse erythroleukemia cells in culture (Lerner et al., 1987), we have not been able to transfer and express the human beta globin gene, our first gene therapy target, into mouse bone marrow cells presumably because it is highly regulated in its expression; therefore, we decided to study the less well-regulated MDR gene; and (3) there are several potential therapeutic uses of adding a highly expressed MDR gene to bone marrow cells; these cells normally express low levels of MDR, and high levels of MDR expression would make the marrow cells more resistant to the toxic effects of drugs which are MDR substrates.

MDR is a transmembrane protein which pumps many naturally-occurring toxic substances out of cells, including drugs commonly used in cancer chemotherapy such as the vinca alkaloids, anthracyclines, etopo-
side and taxol (Pastan and Gottesman, 1991). When a highly expressed MDR gene is transferred to transgenic mice, these mice become resistant to the toxic effects on the white blood cell counts of exposure to these drugs (Galski et al., 1989; Mickisch et al., 1992). This provides a rationale for trying to introduce an MDR gene into the marrow cells of live mice as a model for the eventual clinical application of permitting cancer patients to receive higher doses of chemotherapy with less toxicity. In addition, MDR gene therapy, if successful, could theoretically have a broader application as a selectable gene system to enrich bone marrow cells for those cells not only expressing the MDR gene, but also a non-selectable gene such as the human beta globin gene. In this scenario, the human MDR and beta globin genes would be transferred on the same piece of DNA into bone marrow cells. When the cells are exposed to an MDR drug like taxol, the transduced cells containing both genes would be enriched by their preferential survival. In this way, the patient’s marrow would now contain an increased number of cells with the desirable transferred genes.

Bone marrow has an intriguing special feature as a target tissue which makes it especially attractive for gene therapy: it contains a small population of cells called hematopoietic stem cells (HSC) which are capable through cell division of both self-renewal, as well as differentiation into more mature blood elements including red cells, platelets, lymphocytes and granulocytes (Sutherland et al., 1989; Moore, 1991; Eaves et al., 1992). There are several methods available to transfer genes into marrow cells (Miller, 1990). Most non-viral means and adenovirus-mediated gene transfer, however, do not result in integration of the gene into the chromosomal DNA. To utilize HSC division and differentiation to spread the transferred gene in marrow, a gene transfer method resulting in integration of the gene is highly desirable. Retroviral gene transfer is the most efficient and widely used method to transfer genes into cells as well as to ensure that the transferred genes are integrated into the host cell genome. For these reasons, we have used retrovirus-mediated gene transfer to study the transfer and expression of the human MDR gene into mouse and human bone marrow cells, especially HSC (Podda et al., 1992; Ward et al., 1994b).

Materials and methods

Retroviral gene transfer To provide safe as well as efficient gene transfer, we have constructed unique so-called packaging or helper cell lines to package genes into retroviral particles (Markowitz, 1988a,b). These packaging lines are NIH 3T3 cells transfected with Moloney leukemia virus (MoLV) genes on bacterial plasmids. GP+E 86 and GP-AM12 are two packaging lines we constructed which, when retroviral vectors containing the MDR gene or other genes are added to them, produce MoLV proteins and high levels of retroviral particles, but without generating replication-competent retrovirus. These packaging lines were made by separating the MoLV gag and pol genes (coding for core proteins and reverse transcriptase, respectively) on one plasmid, and the env gene (coding viral envelope protein) on a separate plasmid. GP+E 86 is an ecotropic packaging line containing an ecotropic envelope gene which only transduces (or infects) mouse cells, while AM12 is an amphotropic line with an amphotropic envelope capable of transducing almost all mammalian cells including human cells. High titer ecotropic and amphotropic MDR producer lines have been isolated by transfecting an MDR retroviral vector into GP+E 86 and AM12 clones, respectively, and selecting resistant clones after exposure to colchicine, an MDR-sensitive drug (Podda et al., 1992; Ward et al., 1994b).

Mouse bone marrow isolation and transduction (Podda et al., 1992) Mouse bone marrow cells were collected after exposure of the mice to 5-fluorouracil to enrich for HSCs. The marrow cells were co-cultured with ecotropic MDR producer cells and injected by vein into recipient mice. The recipients were irradiated with 1000–1100 rads to ablate their marrow prior to donor marrow infusion (Hesdorffer et al., 1990). After transplantation, blood samples were obtained from the tail vein and analyzed for MDR gene transfer and expression.

Human bone marrow isolation and transduction (Ward et al., 1994b) Human bone marrow was obtained from donors undergoing marrow harvests prior to autologous bone marrow transplantation (ABMT). Two procedures were used to obtain CD34+ cells which contain the bulk of HSCs; isolation of these cells provides a 50–100 fold purification of HSC. In one procedure, marrow was first isolated using Ficoll gradient centrifugation. These cells were then sub-