Chapter 11

Ex Vivo Gene Transfer into Hepatocytes

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Abstract

Ex vivo gene transfer into hepatocytes could serve several purposes in the context of gene therapy or cell transplantation: (1) isolated hepatocytes can be transduced in culture with therapeutic genes and then transplanted into the recipient; (2) marker genes can be introduced for subsequent identification of transplanted cells and their progeny; (3) gene transfer can be used for conditional immortalization of hepatocytes for expansion in culture; (4) immunomodulatory genes can be transferred into hepatocytes to prevent allograft rejection. Gene transfer into cultured hepatocytes can be achieved using DNA that is not incorporated into recombinant viruses. In such systems, transgene integration into the host cell genome can be enhanced using transposon systems, such as “sleeping beauty.” In addition to using the conventional reagents, such as cationic liposomes, DNA transfer into hepatocytes can be achieved by Nucleofection® or special hepatocyte-targeted carriers such as proteoliposomes containing galactose-terminated glycoproteins (e.g. the F protein of the Sendai virus). Alternatively, genes can be transferred using recombinant viruses, such as adenoviral vectors that are episomal or retroviral vectors (including lentiviruses) that permit integration of the transgene into the host genome. Gene transfer using lentiviral vectors has been achieved in both attached and suspended hepatocytes. Transduction efficiency of lentiviral vectors can be enhanced using magnetic nanoparticles (Magnetofection®).

Key words: Gene transfer, ex vivo, sleeping beauty, nucleofection, F-virosome, lentiviral vectors, magnetofection.

1. Introduction

Transferring genes into isolated hepatocytes could enhance the scope of hepatocyte transplantation. Some examples of the potential uses of ex vivo gene transfer are discussed below to illustrate that the choice of methods to transfer the transgene depends on the ultimate goal of the procedure.
1.1. Objectives of Ex Vivo Gene Transfer into Hepatocytes

1.1.1. Ex Vivo Gene Therapy

This procedure consists of isolating hepatocytes from a patient or a mutant animal carrying a liver-based inherited disease, transducing the cells in culture with a therapeutic gene and then transplanting the phenotypically corrected cells back into the donor. Since the hepatocytes are autologous, this approach circumvents the need for immunosuppression of the host. Long-term efficacy of this strategy requires integration of the transgene into the host genome.

Hepatocytes from a resected liver segment from low-density lipoprotein (LDL) receptor-deficient rabbits (Watanabe heritable hyperlipidemic rabbit) have been transplanted after ex vivo transduction with the low-density lipoprotein receptor (LDLR) gene using recombinant Moloney’s murine leukemia virus (MuLV) vectors (1). This study and the subsequent clinical trial in human subjects (2) with familial hypercholesterolemia had only a minor metabolic effect, which was not sufficient for clinical benefit. Several technical issues limited the success of the procedure. (i) Because cultured primary hepatocytes do not proliferate significantly and have a limited life span in culture, it was not possible to select the transduced cells prior to transplantation. Therefore, the success of the procedure was dependent primarily on the efficiency of transduction. Oncoretroviruses, such as MuLV, require cell division for integration into the chromosome. Despite the use of growth factors in the media, there was only a minor degree of mitosis of hepatocytes in culture. Thus, the efficiency of transduction was limited. (ii) The number of hepatocytes that can be safely transplanted in a single procedure is limited. As no preparative maneuver had been employed to promote preferential proliferation of the transplanted cells in the host liver, the total number of engrafted phenotypically corrected hepatocytes was quite small. Nonetheless, these studies demonstrated that the procedure can be performed safely and delineated the problems involved in this approach, which has stimulated further research, addressing each hurdle as described below.

Vectors, such as those based on immunoretroviruses (lentiviruses) and plasmids that are transposition competent, exhibit a high efficiency of integration in non-dividing cells. Substitution of the oncoretroviral vectors with these vectors could provide a high level of gene transfer into primary hepatocytes, enabling successful ex vivo gene therapy, without the need for prior selection of the transduced cells. New development in the area of hepatic repopulation with transplanted hepatocytes, such as those based on controlled irradiation of the host liver and the use of hepatocyte