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NONVIRAL GENE TRANSFER TO THE LIVER

Baru M, Axelrod JH, Nur I. Liposome-encapsulated DNA-mediated gene transfer and synthesis of human factor IX in mice. Gene 1995;161:143-150.

ABSTRACT

Hemophilia B is an X-chromosome-linked recessive disorder that is caused by a deficiency of biologically active clotting factor IX (FIX). In this work, liposomes (Lip) were used for nonviral, *in vivo* gene transfer of the human *FIX* gene into mouse organs. Plasmid DNA, containing the human *FIX* cDNA under the control of the Moloney murine leukemia virus (MoMLV) long terminal repeat (LTR), was encapsulated in 1- to 2-μm multilamellar Lip composed of egg phosphatidylcholine (EPC). The percentage of Lip-associated DNA was 47%, and 72% of the Lip DNA was protected from DNase I digestion. The Lip-encapsulated (Len) DNA was injected intravenously into BALB/c mice, and at various times postinjection, various tissues were examined for the presence of the exogenous DNA. Plasmid DNA was detected by Southern blot analysis mainly in the liver and spleen, but small amounts were also detected in the lungs, heart, and kidneys. The plasmid DNA was retained in mouse liver cells for at least 7 days postinjection, and remained in an episomal state. The levels of human FIX protein in the mouse plasma were 190 to 650 pg per milliliter for 2 to 7 days postinjection. Treatment of mice with chloroquine (Cq) and colchicine (Cc) prior to Lip injection significantly increased the amount of plasmid DNA found in the liver cells, as well as the level of human FIX in the plasma. These results demonstrate the potential use of Len DNA for gene transfer into liver and spleen, and for gene therapy of inherited and acquired disorders.

COMMENTS

The transfer of factor IX complementary DNA (cDNA) to reticuloendothelial cells by injection of a nonviral vector, namely liposome capsules, has been achieved.[1] In general, gene transfer to somatic cells provides the opportunity to ameliorate and possibly cure inherited diseases such as the hemophilias.[2] Transfer is achieved by a vector or gene delivery system that may be targeted to specific cell types. Transduction (insertion of a vector into cells) can be performed *in vitro* with the expansion of modified, autologous cells in culture and transplantation to the host. Alternatively, *in vivo* transduction involves direct injection or infusion of vectors. Both approaches have had limited success in animal models of hemophilia B. A wide variety of both vector and target cell systems remains under active investigation by several laboratories. There is as yet no single approach that appears effective long-term and is known to be safe for patients.

Safety of viral-derived vector systems is a particularly relevant issue. First-generation recombinant adenoviruses have associated toxicity, particularly immunogenicity resulting in inflammation of transduced tissues *in vivo.* Recent improvement of retroviral vectors to transduce resting as well as dividing cells[3] is achieved by adding both considerable genomic sequence of the human immunodeficiency virus and a substituted envelope protein that markedly stabilizes the viral particle. Efficiency of gene transfer with this construct *in vivo* has not yet been reported, and a number of safety and ethical concerns remain. Although this modified retrovirus vector is mutated to prevent replication, it would require fewer recombinant events (e.g., with endogenous retroviral sequences within the human genome) to revert to a functional acquired immune deficiency syndrome–like virus than would other retroviral vectors.

Several approaches have targeted factor IX cDNAs or minigenes to the liver. Hepatocytes are the normal site of synthesis for this clotting factor. Retroviral factor IX constructs infused via a portal vein catheter remain active in expressing canine factor IX for up to 2 years in hemophilia B dogs.[4] However, circulating factor levels are subtherapeutic, and a partial hepatectomy (or another form of liver injury) is necessary to induce cell division required for retroviral integration into the host cell's genome. Therapeutic and even higher-than-normal factor IX levels are attainable in dogs with severe hemophilia B[5] or normal mice[6] following transduction with adenoviral vectors. To prevent host clearance of episomal, adenoviral transduced cells and/or to promote tolerance to allow repeat injections, some form of immunomodulation is required.[7] The host is then at some risk of enhanced susceptibility to pathogenic adenoviruses or of other effects of chronic (or at least transient, when administered concurrently) immunosuppressive therapy. The latter may not be apparent for many years as seen with a late increased incidence of second neoplasms in patients who receive alkylating agent chemotherapy.

It is important to note that hemophilic patients have a lifelong bleeding tendency that can be managed, at least intermittently, with replacement therapy. Furthermore, totally recombinant (synthetic) preparations of factor VIII and factor IX are now under clinical trials. This underscores the necessity for extremely safe methods of gene therapy as an alternative for managing patients with hemophilia or preventing bleeding episodes. To avoid risks inherent with vector constructs including retroviral, adenoviral, or other partial viral genomes, nonviral vectors have been constructed and examined.

Polylysine tightly conjugates DNA and, with adenoviral particles, for example, allows targeting primarily to hepatocytes when these complexes are infused. For factor IX, such conjugates have transduced fibroblasts *in vitro*[8] and primary hepatocytes *in vitro.*[9] More recently, these types of strategies have been used to produce therapeutic factor IX from the livers of rats.[10] Although the mechanism for DNA loss is different, like adenoviral vectors, problems with transient expression are apparent. A second strategy for nonviral vectors is algenate capsules, but these heterogeneous particles are inefficient in transferring factor IX to mice.[11] A third approach is to deliver DNA in liposome particles. These have theoretic advantages of being neither toxic nor immunogenic. Furthermore, as opposed to retroviral, and more like adenoviral vector systems, large DNA constructs such as factor IX mini-genes or complete factor VIII cDNA can be used to transduce cells.

The study by Baru et al.[1] represents an important initial step in developing a reasonable system to protect and insert factor IX DNA into cells by liposome particles. As predicted, insertion is predominantly into reticuloendothelial cells, and the organ distribution is liver and spleen with lesser amounts in other organs. This contrasts with polylysine-based conju-

gates that have often been targeted to primarily transduce hepatocytes. In their study, Baru et al., obtained very low levels of plasma factor IX (<1 ng/mL) for up to 1 week. Thus, much greater levels of gene expression will be needed, as well as developing a means to stabilize or safely give repetitive infusions of the vector. Furthermore, as opposed to factor IX, proteins such as factor VIII that have limited stability and are easily inactivated by proteolysis may have significant additional problems being expressed by reticuloendothelial cells as opposed to hepatocytes; factor IX, however, is less susceptible to proteolytic degradation than factor VIII.

Regardless of the vector, a potential problem is an immune response to the transgene. For patients with severe hemophilia, it is possible that even if an immune response were to occur, the low level of continuous expression of the deficient factor would create immune tolerance, a condition achieved in about 75% of hemophilia A patients who develop clinically significant alloantibody inhibitors. Expression would thus abrogate most immune responses.

The next steps in evaluating the potential for liposome encapsulation in transducing animals with the factor IX gene will be to improve formulations for efficient cell-specific targeting, develop means for efficient and stable DNA delivery to the nucleus, and insert constructs that are more efficient producers of factor IX *in vivo,* such as mini-genes used in viral vectors to transduce hepatocytes[6] or myoblasts.[12] Persistence of human factor IX expression for several weeks would also be necessary if repeated liposome injections were to be considered preferable to prophylactic infusions with recombinant factor. The necessity for chloroquine and colchicine treatment[1] and potential minor toxicity from these drugs remains another issue to be addressed should liposome transduction provide higher levels of factor IX for longer periods of time. It remains to be determined if the efficiency and persistence of nonviral transduction can be enhanced to approach that seen with viral vectors. If so, nonviral vectors could provide a safer alternative for gene transfer.

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PLASTICITY OF THE HEPATOCYTE PHENOTYPE *IN VITRO:* COMPLEX PHENOTYPIC TRANSITIONS IN PROLIFERATING HEPATOCYTE CULTURES SUGGEST BIPOTENT DIFFERENTIATION CAPACITY OF MATURE HEPATOCYTES

Block GD, Locker J, Bowen WC, Petersen BE, Katyal S, Strom SC, Riley T, Howard TA, Michalopoulos GK. Population expansion, clonal growth, and specific differentiation patterns in primary cultures of hepatocytes induced by HGF/SF, EGF and TGFα in a chemically defined (HGM) medium. J Cell Biol 1996;132:1133-1149.

ABSTRACT

Mature adult parenchymal hepatocytes, typically of restricted capacity to proliferate in culture, can now enter into clonal growth under the influence of hepatocyte growth factor (scatter factor) (HGF/SF), epidermal growth factor (EGF), and transforming growth factor α (TGFα) in the presence of a new chemically defined medium (HGM). The expanding populations of hepatocytes lose expression of hepatocyte specific genes (albumin, cytochrome P450 IIB1), acquire expression of markers of bile duct epithelium (cytokeratin 19), produce TGFα and acidic FGF, and assume a very simplified morphologic phenotype by electron microscopy. A major change associated with this transition is the decrease in ratio between transcription factors C/EBPα and C/EBPβ, as well as the emergence in the proliferating hepatocytes of transcription factors AP1 and NFκB. The liver associated transcription factors HNF1, HNF3, and HNF4 are preserved throughout this process. After population expansion and clonal growth, the proliferating hepatocytes can return to mature hepatocyte phenotype in the presence of EHS gel (Matrigel). This includes complete restoration of electron microscopic structure and albumin expression. The hepatocyte cultures however can instead be induced to form acinar/ductular structures akin to bile ductules (in the presence of HGF/SF and type I collagen). These transformations affect the entire population of the hepatocytes and occur even when DNA synthesis is inhibited. Similar acinar/ductular structures are seen in embryonic liver when HGF/SF and its receptor are expressed at high levels. These findings strongly support the hypothesis that mature hepatocytes can function as or be a source of bipotential facultative hepatic stem cells (hepatoblasts). These studies also provide evidence for the growth factor and matrix signals that govern these complex phenotypic transitions of facultative stem cells which are crucial for recovery from acute and chronic liver injury.

COMMENTS

The two major epithelial cell types that compose the adult rodent liver (hepatocytes and bile duct epithelial cells) are long-lived, and under normal physiological conditions do not demonstrate appreciable levels of mitosis or apoptosis.[1,2] Nonetheless, the adult rodent liver retains the capacity for complete and rapid renewal in response to cell loss via proliferation of both hepatocytes and biliary epithelial cells.[1-4] The