


5. Schwartz ME, Sung M, Mor E, Fisher A, Popescu I, Fiel I, Shneider P, et al. 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) immune response. The latter may not be apparent for many years as seen with a late indication of human FIX cDNA in cells by liposome particles. As predicted, the transfer of factor IX complementary DNA (cDNA) to reticuloendothelial cells by injection of a nonviral vector, namely liposomal capsules, has been achieved.

6. Laron DNA for gene transfer into liver and spleen, and for in vivo retroviral vectors. To prevent host clearance of episomal, adenoviral transduced cells and/or to promote tolerance to allow repeat injections, some form of immuno-modulation is required. 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) immune response. The latter may not be apparent for many years as seen with a late indication of human FIX cDNA in cells by liposome particles. As predicted, the transfer of factor IX complementary DNA (cDNA) to reticuloendothelial cells by injection of a nonviral vector, namely liposomal capsules, has been achieved.

7. Liposome-encapsulated DNA-infused via a portal vein catheter remain active in expressing canine factor IX for up to 2 years in hemophilia B dogs. 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 or normal mice following transduction with adenoviral vectors.

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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 getting, develop means for efficient and stable DNA delivery 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.

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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 or myoblasts. 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 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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REFERENCES

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


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 (TGF-α) in the presence of a new chemically defined medium (HGM). The expanding populations of hepatocytes lose expression of hepatocyte specific genes (albumin, cytochrome P450 IB1), acquire expression of markers of bile duct epithelium (cytokeratin 19), produce TGF-α 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 NFkB. 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