MINI REVIEW

Gene therapy of liver diseases: A 2011 perspective

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Available online 22 July 2011

Summary  Liver diseases including inherited metabolic disorders, chronic viral hepatitis, liver cirrhosis and primary and metastatic liver cancer constitute a formidable health problem because of their high prevalence and the important limitations of current therapies. Gene therapy, a procedure based on the transfer of therapeutic genes to tissues, has been used since the 1990s as a new approach to treating a number of incurable conditions. After a period of lights and shades recent success in treating several devastating diseases like inherited immune deficiency disorders, beta-thalassemia, or inherited blindness appear to herald a new era where gene therapy can be listed among standard therapy options for a wide variety of human conditions. In this review, we provide information illustrating the potentiality of gene therapy in the management of liver diseases lacking other effective therapies.

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Gene therapy consists of the introduction of genetic material into cells for a therapeutic purpose. The introduction of foreign genes inside the target cells is facilitated by the use of molecular constructs, named vectors, that permit the access of the gene to the intracellular milieu and protect the transgene from degradation [1]. Each application requires the use of a vector and a transgene (or transgenes) selected according to the therapeutic strategy used. Two approaches for gene transfer can be employed: in vivo gene therapy, which is accomplished by direct delivery of vectors into tissues or blood, and ex vivo, where cells (e.g. hepatocytes, dendritic cells, lymphocytes, macrophages, hematopoietic stem cells, endothelial progenitor cells . . .) are isolated from the patients to be genetically modified in vitro and then transplanted back into the same individual. This strategy combining cell and gene therapy holds considerable promise for the treatment of a diversity of pathological processes [1].

Gene therapy vectors can be categorized into viral and non-viral. Taking advantage of natural infectivity of viruses, viral vectors are produced by deleting some or all viral genes which are replaced by the sequences encoding the therapeutic molecule. Each viral vector system is characterized by an inherent set of properties which determines its suitability for specific gene therapy applications. Some genetic disorders can be compensated by transduction of a limited proportion of liver cells with a long-term expression vector encoding the correct version of the defective gene. Other pathological processes, however, might require gene transfer to a large fraction of target cells and transient or regulated gene expression. Cloning capacity, toxicity and immunogenicity, as well as the feasibility and cost of large-scale production, is also a feature to be considered when choosing the appropriate vector [1]. The number of viruses that are under development as gene therapy vectors is
steadily increasing, but at present there are three main clinically applicable vectors for liver transduction: adenoviruses, adeno-associated viruses (AAV) and lentivirus. The liver, being a solid, well vascularized and readily accessible organ, is particularly amenable to be transduced by gene therapy vectors. A great diversity of liver diseases could potentially be treated by gene transfer procedures, including liver cirrhosis, viral hepatitis, primary and metastatic liver cancer, and inherited liver disorders [2,3]. In this review, we will make a brief consideration of each of these applications.

Liver-directed gene therapy for inherited metabolic diseases

For most of inherited metabolic liver diseases no effective therapy is available except liver transplantation, which is hampered by donor shortage, cost, surgical risks and long-term immunosuppression. Thus, safer and more efficient therapies are greatly needed. Recent bibliography shows that complete phenotypic correction of many inherited metabolic disorders affecting the liver can be achieved in animal models using one of the most promising gene therapy vectors, the AAV vector. Acute intermittent porphyria (AIP) [4], phenylketonuria [5], Ornithine transcarbamylase (OTC) deficiency [6], hereditary tyrosinemia [7], familial hypercholesterolemia (Kasim SH), or Crigler-Najjar [8] and many others, can be cured using AAVs that provided long-term correction. Furthermore, other genetic disorders not affecting specifically the liver, but that can be genetically corrected from the liver-like hemophilia have been treated using AAV vectors [9]. In fact, the first clinical trial using an AAV vector to transduce the liver was performed in hemophilia patients. This first clinical trial failed to maintain curative level of factor IX for more than 1 month because of activation of T-cell immunity against the viral capsid [9]. In a second trial which is currently ongoing the administration of a different AAV serotype expressing factor IX together with corticoids lead a prolonged expression of the transgene [10].

As shown in the first hemophilia clinical trial targeting the liver one of the main problems face by gene therapy is the host immune response directed against the transgene product or against the vector particles. This severely impairs the efficiency of gene transfer, and precludes long-term transgene expression after in vivo gene delivery. This problem has been elegantly addressed by Brown et al. [11] avoiding transgene expression in antigen presenting cells (APCs) by using a cell type specific promoter together with the incorporation in the messenger RNA sequence of APC-specific micro RNA target sequences. Employing this strategy Schmitt et al. [12] were able to normalize serum bilirubin in a Crigler-Najjar animal model. Other authors have achieved long-term transgene expression by employing ex vivo transduced cells as vehicles for the transgene. Using this approach, it has been shown that mesenchymal stem cells obtained from adipose tissues and transduced with an AAV vector expressing human alpha-1 antitrypsin resulted in long-term transgene expression in the liver [13].

Gene therapy for viral hepatitis B and C

Gene therapy can be used to either prevent or to treat HBV and HCV viral infections. Genetic vaccines aim to stimulate the host’s immune response against viral antigens, by injecting into the individual gene sequences encoding viral products accompanied or not by sequences coding for immunostimulatory cytokines. Other strategies based on the injection of autologous dendritic cells engineered ex vivo to express viral proteins can also be used as powerful means to activate antiviral immunity [14].

Several clinical trials of genetic vaccines against HBV and HCV are ongoing or planned, as summarized in Table 1 [15–24]; however, little information is currently available regarding the antiviral efficacy of these therapies. In a recent clinical trial, patients with chronic HBV infection being on lamivudine treatment were immunized with a vaccine consisting of plasmids encoding both HBV antigens and human IL-12. After long-term follow-up period, 50% of the vaccinated patients demonstrated undetectable viremia, whereas no changes in serum viral loads were observed in the remaining patients [18]. Overall, the results of this trial are encouraging and demonstrate the potential of genetic vaccination in combination with antiviral treatment. Interestingly, Okairos Industry has started three phase I clinical trials (one prophylactic and two therapeutic) to test the safety of two different serotypes of adenovirus: human adenovirus serotype 6 (Ad6) and simian adenovirus serotype 3 (AdCh3) found at low sero-prevalence in human populations. The recombinant adenovirus express an HCV non-structural proteins with a genetically inactivated polymerase gene, NSmut [25]. Furthermore, a phase I trial carried by Tripep AB using a DNA-based vaccine administrated by electroporation demonstrated viral load reductions by Up to 99.7%, phase II trial is now ongoing [22]. A recent report shows that strong T-cell immunity can be elicited in mice by ex vivo targeting of adenoviral vectors encoding NS3 from HCV using an adapter hybrid molecule containing both Coxakie-Adenovirus Receptor (CAR) molecule AR and CD40L which simultaneously facilitates cell transduction and strongly activates dendritic cells [26]. A clinical trial using this approach as a therapeutic vaccination for chronic HCV infection will start this year.

Recent GT approaches have been designed to directly target viral replication. One such approach is based on the use of interference RNA (RNAi), in which genes are silenced in a sequence-dependent manner. RNAi has already been investigated in models of hepatitis viral infections with great success. It was demonstrated that siRNAs can alter the course of HBV and HCV infection by mediating viral RNA degradation, resulting in inhibition of viral RNA translation and replication. Treatment with plasmid or virus-encoded shRNAs for specific targeting of HBV sequences reduced the levels of serum HBV surface antigen (HBsAg) and HBV core antigen (HBCAg), and decreased viral genomic DNA and viral RNA transcription in the liver of mice [27]. The 5′ untranslated region of the HCV viral genome, as well as the NS3 and NS5B genes, have been demonstrated to be effective targets for synthetic siRNAs or vectors expressing shRNAs [27]. However, a common problem in these studies is viral escape that derives from the high mutation rate of the viral genome [27]. This problem can be circumvented by targeting host genes
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<td>HCC and liver metastasis</td>
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<td>HCC and liver metastasis</td>
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<td>Therapeutic</td>
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required for HCV replication. Numerous cell proteins have been identified that appear to be essential for a productive HCV infection. Provided the inhibition of these host proteins does not cause adverse effects in patients, these proteins could represent ideal targets. Among host cell factors, endogenous regulatory miRNAs (miRNA) constitute interesting potential targets; for example, the liver-expressed miR-122 binds to two target sites in the 5′ untranslated region of HCV, resulting in upregulation of viral RNA levels. Taking advantage of this function, a recent study has demonstrated that treatment of chimpanzees chronically infected with HCV with a modified oligonucleotide complementary to miR-122 resulted in long-lasting suppression of HCV viremia [28]. Treated animals exhibited improved liver pathology without side effects, and no evidence of viral resistance was observed, suggesting that targeting host factors essential for HCV replication has a high therapeutic potential. The main limitation of strategies based on genetic interference of viral replication is that the inhibitory molecule must reach a high percentage of the infected hepatocyte in order to produce a significant effect. Furthermore, shRNA overexpression from viral vectors can trigger cytokotoxicity leading to organ failure and lethality in mice and rats due to saturation of endogenous cellular RNAi factors [29].

**Gene therapy of liver cirrhosis**

Hepatic cirrhosis is the result of a sustained liver damage leading to altered organ architecture with development of regenerative nodules surrounded by fibrous tissue. The main clinical problems of cirrhotic patients are those derived from hepatocellular insufficiency and portal hypertension. Besides, supportive therapy for complications, advanced liver cirrhosis can only be treated at present by liver transplantation. However, donor limitations, economic cost, need for long-term immunosuppression, age of the patient and the presence of conditions contraindicating the procedure severely limit the applicability of transplantation to a high proportion of cirrhotic patients. Alternative therapies for liver cirrhosis are one of the most urgent needs in current hepatological practice. The possibility of transducing the cirrhotic hepatic tissue with genes encoding hepatoprotective and antifibrogenic factors offers an attractive perspective in the management of liver cirrhosis. In pioneering studies, Ueki et al. in 1999 showed that the transduction of the cirrhotic liver with vectors that expressed Hepatocyte Growth Factor (HGF) caused an impressive diminution of fibrosis and a significant improvement of the hepatocellular function. Another transgene of potential interest for the treatment of the cirrhosis is the Insulin Growth Factor I (IGF-I). This cytoprotective and anabolic hormone is produced by hepatocytes under stimulation by Growth hormone (GH) and its levels are profoundly decreased (or undetectable) in advanced cirrhosis. It has been recently shown [30,31] that the transduction of the liver of cirrhotic rats with a long-term expression vector encoding IGF-1 resulted in a marked reduction of fibrosis together with a significant improvement of liver function. The transgenic IGF-1 produced by hepatocytes in the cirrhotic liver acted on hepatic stellate cells (HSCs), where IGF-IR is mainly expressed, promoting downregulation of profibrogenic factors (including TGF-β and PDGF), deactivation of HSCs, upregulation of matrix metalloproteases, induction of cytoprotective factors such as HGF and attenuation of inflammation [30,31]. Clearly, clinical trials are required to assess the ability of IGF-1-based gene therapy to modify the biology of liver cirrhosis with signals promoting hepatocyte survival and restraining scar formation. As liver cirrhosis is a preterminal condition, IGF-1 being an antiapoptotic growth factor may favour tumor development. However, recent transcriptome analysis of peritumoral cirrhotic liver indicates that it is precisely the lack of IGF-1 what associates with higher risk or HCC recurrence after curative resection [32]. On the other hand, since hepatocarcinogenesis is linked to fibrosis and persisting inflammation, the antiinflammatory and antifibrogenic effects of IGF-1 might in fact reduce tumor development.

**Gene therapy of liver cancer**

Gene therapy is a promising therapy for both primary and metastatic live cancer. Hepatocellular carcinoma (HCC) is the most prevalent primary liver tumor followed by cholangiocarcinoma (CC). In most cases, HCC is diagnosed beyond the limits for curative therapy (resection, ablation, transplantation) and when this occurs arterial chemoembolization or radioembolization can be performed in selected patients. When these approaches are not applicable, or the tumor progress following therapy, the only option is targeted biological therapy with sorafenib, which offers a very modest prolongation of survival at the cost of side effects and economic burden. Together with HCC and CC, metastatic tumors to the liver such as colorectal cancer or neuroendocrine tumors may kill, mainly because of intrahepatic spread. When these tumors become resistant to current therapy constitute situations where liver-directed gene therapy might be of utility.

Strategies for cancer gene therapy include transfer of cancer suppressor genes, blockade of oncogenes, use of suicide genes, stimulation of antitumor immunity, modification of tumor microenvironment, inhibition of angiogenesis and virotherapy (Fig. 1). Gene therapy can be used combined with other therapies such as chemotherapy. It has been shown that modification of tumor microenvironment with chemotherapy enhances the antitumor effect of gene therapy based on the use of immunostimulatory molecules [33].

**Restoration of tumor suppressor genes**

Several approaches to restore p53 function have been investigated for the treatment of HCC. A first-generation adenoviral vector expressing wild-type p53, Gendicine, has been commercially licensed in China, and has been used in several clinical trials for the treatment of HCC in combination with other treatments such as chemotherapy or chemoembolization [34]. The benefit of this vector when administered alone is controversial, the number of patients is low and there is not information available about the details of the trials, but promising results were obtained recently when given in combination with fractionated stereotactic radiotherapy [35].
Figure 1 Strategies for liver restoration of tumor suppressor genes, immunotherapy, virotherapy and modulation of tumor microenvironment.

**MiRNA**

Altered miRNA expressions have been observed in HCCs. The different expression profiles of miRNAs in HCC suggest that miRNAs may serve as either novel potential targets acting directly as oncogenes or therapeutic molecules working as tumor suppressor genes. There is a reduced abundance of miR-26 in human HCC compared with paired noncancerous tissues. It has been shown that an AAV vector encoding this miRNA caused selective apoptosis of cancer cells and inhibited the progression of tumors in a c-myc transgenic model without toxicity [36]. Recent findings indicate that restoration of other miRNAs such as miR-122, miR-195 and miR-101 (which are downregulated in a significant proportion of human HCCs) could have therapeutic effect against HCC as they control the expression of genes implicated in oncogenic pathways [37]. Interestingly, a combination of miRNAs can be expressed simultaneously using currently available vectors opening a new path for drug development in liver cancer.

**Expression of pro-drug converting genes**

The aim of suicide gene therapy is to enable, selectively, the transfected cell to transform a pro-drug into a toxic metabolite, resulting in cell death. The herpes simplex type 1 thymidine kinase (HSV-TK) catalyzes the phosphorylation of ganciclovir, causing the accumulation of toxic metabolites in the cells. Inoculation of a similar vector (ADV-TK) in the peritoneum of HCC patients at the moment of liver transplantation and subsequent treatment with ganciclovir achieved a remarkable increase in recurrence-free survival compared with liver transplantation alone [38]. In patients without vascular invasion, overall survival was 100% at 3 years. Therefore, the same approach was unable to eradicate bulky disease but showed a promising effect as an adjuvant to conventional therapies [39]. Other enzyme/pro-drug combinations under study are cytochrome p450/cyclophosphamide, Nitroreductase/dinitrobenzamide CB or cytosine deaminase gene (CD)/5-fluorocytosine (5-FC) systems [40].

**Immunotherapy**

Stimulation a protective immune response against cancer cells is an appealing goal for the treatment of all kind of tumors. In the case of HCC, this is especially desirable, taking into account that most patients suffer an underlying liver cirrhosis and are prone to develop new tumors. Intratumor expression of immunostimulatory cytokines such as interleukins, IFN-α or γ, TNF-α and GM-CSF are effective in eradicating HCC in mouse and rat models [2].
These approaches are advantageous compared with the systemic administration of the recombinant proteins, in terms of efficacy and toxicity. A clinical trial using intra-tumor administration of a first-generation adenoviral vector expressing interleukin 12 (AdIL-12) demonstrated the feasibility and safety of this approach. Patients with primary and metastatic liver cancer received a single administration of the vector. Stimulation of the immune system could be detected, but no complete responses were observed, probably due to the low and transient expression of the cytokine [41]. Similar results were obtained after intratumoral administration of dendritic cells infected with AdIL-12 [42]. This prompted the development of improved vectors. A high-capacity (gutless) adenoviral vector carrying a liver-specific inducible system for the expression of IL-12 is able to maintain controlled and sustained cytokine production [43]. The efficacy of this approach against animal models of cancer and hepatitis B has been demonstrated, and its clinical application awaits optimization of manufacturing protocols to produce this kind of vector [33,44].

**Oncolytic virotherapy**

Oncolytic virotherapy is based on the capacity of some viruses, either naturally or after genetic modifications, to replicate preferentially in cancer cells and cause their destruction. In principle, they will undergo multiple rounds of replication, virus release and re-infection in malignant cells. Oncolytic viruses (OV) which are based on adenovirus, herpes simplex type I (HSV-1), vesicular stomatitis virus (VSV), vaccinia virus (VV) or Newcastle disease virus (NDV) have been demonstrated to mediate antitumor effects in preclinical animal models of HCC [45]. The ONX-015 oncolytic adenovirus showed a moderate antitumor effect in animal models, in part because the E1B 55K deletion impaired its cytopathic potency also in cancer cells. Treatment of HCC patients with this virus was well tolerated, but its efficacy as monotherapy was very limited [46].

For enhancing the antitumor effect mediated by OVs, different strategies are now being tested such as arming the virus with therapeutic genes (e.g., cytokines, angiogenic molecules such as endostatin, and others [47–51]). In a recent phase I–II clinical trial, three patients with HBV-associated advanced HCC were treated with an oncolytic VV expressing GM-CSF (named JX-594). JX-594 was designed to selectively replicate in cancer cells and to destroy malignant cells presenting cell-cycle abnormalities and epidermal growth factor receptor (EGFR)-ras pathway activation. JX-594 showed effectiveness against liver cancer in a phase II clinical trial and will move into a phase III trial later this year. In the phase II study, 18 of 24 patients survived at least 12 months; with standard treatment, only about half of patients survive one year. Furthermore, significant decreases in HBV DNA concentration of 70–91% from baseline were noted [49]. The authors also provided insight into the underlying mechanism of action by demonstrating that the OV was able to shutdown the blood flow to the tumor. This effect was associated with a reduction in circulating VEGF and with the induction of cytokines known for mediating antivascular effects (i.e., TNF-α and IFN-γ). The last results obtained with this virus have been presented at the 46th Annual Meeting of the European Association for the Study of the Liver (EASL) 2011, showing that 66% of the patients exhibited significant tumor necrosis and decreased tumor density and 20% also exhibited objective response by Response Evaluation Criteria in Solid Tumor (RECIST) criteria, including two complete responses upon long-term follow-up. Twenty patients (57%) had stable disease as defined by RECIST criteria [52].

**Modulation of tumor microenvironment**

Angiogenesis is a key issue for the maintenance of tumor growth, particularly for HCC, which is characterized by marked hypervascularization. Tumor expression of VEGF has been shown to correlate with tumor invasiveness and prognosis in patients with HCC [53]. VEGF is an important molecular target for antiangiogenic therapy. Studies in animal models have demonstrated the efficacy of antiangiogenic agents such as anti-VEGF antibody and antagonists of VEGF receptors in suppressing hepatocarcinogenesis and growth of HCC [54]. Wnt signaling, in addition to direct effects on tumor cells, is involved in the organization of tumor microenvironment. Recently, we have shown that adenoviral expression of Wnt antagonists inhibits tumor angiogenesis and control tumor growth [55]. Furthermore, modulation of tumor microenvironment enhances the antitumoral activity of conventional chemotherapeutic. Down regulation of hypoxia-inducible factor (HIF)-1 using antisense molecules enhance the antitumoral activity of Doxorubicin (Dox) in an HCC animal model [56]. Genetic immunotherapy have been also shown to modify tumor microenvironment enhancing the effect of chemotherapy, overexpression of the melanoma differentiation associated gene-7 (MDA-7)/IL-24 or IL-12 enhance the antitumoral efficacy of Dox and oxaliplatin, respectively [33,57].

**Gene therapy of Acute Liver Failure (ALF)**

Acute liver failure (ALF) is a clinical condition that develops within a few weeks of the onset of symptoms in patients without preexisting liver disease. Different etiologies may underlie this syndrome but extensive liver damage by proinflammatory cytokines in the context of severe viral hepatitis or autoimmune reactions is a relevant mechanism for liver cell death in a significant proportion of patients. ALF remains one of the most challenging of all critical conditions and new therapeutic options are urgently needed. One of the potential strategies to prevent ALF is to target the key molecules participating in the death of hepatocytes, such as Fas/FasL, TNFα, Trail or TGFβ [3]. Treatment with adenovirus-mediated dominant negative form of the Fas-associated death domain (FADDn), a downstream signaling molecule for Fas and TNFRs, inhibited the TNF/Galactosamine mediated hepatocellular apoptosis and significantly lowered the levels of serum transaminase in mice [58]. Induction of TGFβ-RiI specific siRNA expression by hydrodynamic injection with the shRNA plasmid inhibited the TGFβ signaling and protected the mice from anti-Fas antibody-induced ALF [59]. Furthermore, hepatic expression of hepatoprotective cytokines like IL-6 or Cardiotrophin-1 (CT-1) abrogated liver injury in differ-
Conclusions and perspectives

Recent technical and scientific advances in gene and cell therapy together with a better knowledge of the molecular mechanism of liver diseases have paved the way for the development of new therapeutic strategies. Abundant preclinical studies have demonstrated that many forms of liver diseases are amenable to treatment with gene transfer methodologies. Genetic corrections of inherited metabolic disorders, genetic vaccines against chronic viral infection and a diversity of gene transfer strategies for the treatment of liver cancer have already reached the clinical arena. These pioneer studies have shown that gene therapy is safe and can afford benefit. Furthermore, promising preclinical data have been obtained in relevant animal models of liver cirrhosis and acute liver failure using gene transfer strategies which deserve consideration for clinical application. In summary, gene therapy is a promising approach which could fill the therapeutic vacuum that presently exists for a diversity of serious liver conditions.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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