Gene therapy for liver regeneration: Experimental studies and prospects for clinical trials

Hussein M Atta

Abstract

The liver is an exceptional organ, not only because of its unique anatomical and physiological characteristics, but also because of its unlimited regenerative capacity. Unfolding of the molecular mechanisms that govern liver regeneration has allowed researchers to exploit them to augment liver regeneration. Dramatic progress in the field, however, was made by the introduction of the powerful tool of gene therapy. Transfer of genetic materials, such as hepatocyte growth factor, using both viral and non-viral vectors has proved to be successful in augmenting liver regeneration in various animal models. For future clinical studies, ongoing research aims at eliminating toxicity of viral vectors and increasing transduction efficiency of non-viral vectors, which are the main drawbacks of these systems. Another goal of current research is to develop gene therapy that targets specific liver cells using receptors that are unique to and highly expressed by different liver cell types. The outcome of such investigations will, undoubtedly, pave the way for future successful clinical trials.
LIVER REGENERATION: BASIC CONSIDERATIONS

Following two-thirds partial hepatectomy, the residual liver lobes enlarge within a week to make up for the mass of the removed lobes. Liver regeneration is carried out by proliferation of all adult liver cells including hepatocytes, sinusoidal endothelial cells, biliary epithelial cells, Kupffer cells and hepatic stellate cells (HSCs). It has been firmly established that mature hepatocytes are not terminally differentiated and that they have an almost unlimited capacity to proliferate, so that the liver can be entirely repopulated by intact hepatocytes that represent 1% of the hepatocyte population.

The molecular mechanisms of liver regeneration can be divided into two critical steps: the transition of the quiescent G0 phase hepatocyte into the cell cycle (priming phase), and progression beyond the restriction point in the G1 phase of the cycle (progression phase). These phases are under separate control; priming by the cytokines tumor necrosis factor (TNF) and interleukin-6 (IL-6), and cell cycle progression by the growth factors hepatocyte growth factor (HGF) and transforming growth factor (TGF)-α. The priming phase does not lead to DNA replication unless the cells can progress through the cell cycle which is accomplished by growth factors. Once hepatocytes pass the G1 restriction point they are irreversibly committed to replication (Figure 1).

The mechanisms that initiate cytokine cascade liver regeneration have not yet been fully identified. It has been proposed that liver injury causes the release of reactive oxygen species and lipopolysaccharide (LPS), which triggers the activation of the complement system. After complement activation, cleavage of C3 or C5 leads to generation of the potant anaphylatoxins C3a and C5a. LPS, C3a and C5a in turn activate the non-parenchymal cells (NPCs) such as Kupffer cells, through the cell surface receptor TLR4 and C3aR and C5aR, which causes activation of the transcription factor nuclear factor (NF)-κB signaling pathway and the production of cytokines such as TNF-α and IL-6. Also, the cytokine cascade can be triggered through the binding of TNF to its receptor TNFR1, which leads to activation of the NF-κB in NPCs, with the production of TNF and IL-6. Thus, the released TNF acts on the same NPCs in an autocrine fashion and on hepatocytes by a paracrine mechanism. Released IL-6 binds to its receptor on hepatocytes and leads to activation of the transcription factor STAT3 (signal transduction and activator of transcription), which translocates to the nucleus where it induces transcription of a number of target genes (Figure 2). The precise role played by each cytokines is, however, debatable. TNF is not a direct mitogen for hepatocytes. It does, however, enhance the mitogenic effects of direct mitogens such as HGF.

Example, it has been shown in stellate cells in culture that TNF and IL-6 activate the transcription factor C/EBPβ (CCAAT/enhancer-binding protein β), which induces HGF mRNA expression. TNF is also involved in the activation of TGF-α. IL-6 has both mitogenic and anti-apoptotic effects on hepatocytes and protects the regenerating liver against ischemic injury. IL-6 has a crucial role in initiating acute phase response in hepatocytes, with the production of many proteins that assist in controlling acute or chronic inflammation.

While cytokines are responsible for the passage of quiescent hepatocytes into the cell cycle (G0 to G1), cell cycle progression is then driven by growth factors, which override a restriction point in the late G1 phase. HGF and ligands of epidermal growth factor receptor (EGFR) are important growth factors that drive cell cycle progression during liver regeneration. Studies have shown that despite the expression of many mitogenic receptors, including receptors for platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF), the only mitogens for hepatocytes are HGF and ligands of EGFR. The family of ligands that bind EGFR, in addition to EGF, includes TGF-α, heparin-binding EGF-like growth factor (HB-EGF), and amphiregulin (AR). Stimulation of the tyrosine kinase receptors for HGF and the EGFR ligands activates numerous intracellular signaling pathways that regulate transcription factors involved in liver regeneration. It is important to mention, with the possible exception of HGF, that complete elimination of a single growth factor does not entirely abrogate liver regeneration.

HGF is the most extensively investigated growth factor for liver regeneration. It stimulates regeneration in normal and injured liver. It is produced by NPCs and stimulates hepatocytes by a paracrine or endocrine mechanism. Following binding to its receptor, cMet, on hepatocytes, it stimulates DNA synthesis. HGF effects are multiple including mitogenic, motogenic, morphogenic and anti-apoptotic effects.

EGFR ligands are direct mitogens for hepatocytes. EGF is continually available to the liver through the portal...
Cytokine and growth factor pathways interact during different phases of liver regeneration. EGF, HB-EGF, AR are mitogens for endothelial cells and bile duct epithelial cells. HB-EGF is produced by endothelial and Kupffer cells and is a key factor for hepatocyte progression through G1/S transition during liver regeneration. AR also contributes to liver regeneration, because mice deficient in AR have deficient liver regeneration. It is likely that the different growth factors have independent but partially overlapping functions in liver regeneration.

Figure 2 Major cytokine and growth factor signals during liver regeneration. NF-κB: Nuclear factor-κB; TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6; EGF: Epidermal growth factor; TGF-α: Transforming growth factor α; VEGF: Vascular endothelial growth factor; FGF: Fibroblast growth factor; HB-EGF: Heparin-binding EGF-like growth factor; AR: Amphiregulin; C/EBP: C/EBP: CCAAT/enhancer-binding protein; HGF: Hepatocyte growth factor.
cific growth factors that promote liver regeneration allow the development of recombinant growth factors and their use to promote liver regeneration\[6,40-43\]. The success of this strategy is hampered by the short half-life of these proteins in the circulation and the need for them to be administered continuously. To overcome this problem, investigators have successfully used gene transfer technology to transfer the genes that encode these growth factors into liver cells.

**GENE THERAPY FOR LIVER REGENERATION: KEY CONCEPTS**

The strategy of introducing genetic material into liver cells to enhance proliferation or to inhibit apoptosis has been employed in experimental liver research for more than a decade. The transferred genetic material can be a natural gene\[44-46\], gene segment\[47\], chimeric gene\[48\], oligodeoxynucleotides (ODN)\[49,50\], or siRNAs. To facilitate transfer (transduction) into cells, the foreign gene (transgene) is packaged into construct named vectors. Gene transfer vectors are classified as either viral or non-viral. Viral vectors provide a powerful means for delivering therapeutic genes to targeted cells due to their high transduction efficiency. They are made replication-defective by deletion of viral genes involved in the replication and pathogenesis of the virus. This allows for the inclusion of non-viral genetic material in the viral genome. The general characteristics of most commonly used vectors are shown in Table 1. The most commonly used viral vectors are retrovirus, adenovirus, adeno-associated virus (AAV), herpes simplex virus, lentivirus and baculovirus. For a gene to be expressed inside a cell, its coding DNA sequence should be linked to an appropriate promoter. These regulatory DNA sequences can be categorized as viral (universal) promoters, which allow transgene expression in most transduced cells, housekeeping promoters, or tissue-specific promoters, which drive gene transcription only in selected cell types\[51\]. Because of their universal activity, viral promoters were components of many first-generation vectors. However, many of the viral promoters, such as the cytomegalovirus (CMV) promoter, are attenuated or completely shut-off in organs such as the liver. In comparison to viral or housekeeping promoters, tissue- or liver-specific promoters direct higher levels of expression in vivo. Successful application of gene therapy depends on the choice of relevant therapeutic genes, appropriate promoters, and effective vectors that allow an adequate level and duration of transgene expression\[52-54\].

Although retroviral vector transfection results in long-term survival of the gene in the transduced cell, its major disadvantage is the risk of insertional mutagenesis as a result of random integration of the virus into the host chromosome. Moreover, the transduction rate after retroviral gene transfer into hepatocytes in vivo is disappointingly low. Efficient retrovirus integration into the host-cell genome requires the active proliferation of target cells with DNA replication and nuclear membrane breakdown during mitosis. Under normal physiological conditions at any given time, only 0.005% of hepatocytes divide. For retrovirus liver transduction, hepatocyte proliferation induced by PH must occur on or about the time of retroviral delivery. To increase gene transfer without hepatectomy, mouse hepatocytes have been transduced in vivo with a recombinant adenovirus that transiently expressed urokinase\[55\], or with recombinant HGF\[56\]. The induced liver regeneration allowed persistent and efficient retroviral-mediated gene transfer in hepatocytes\[55,56\].

Adenoviral vectors are the most investigated vectors in animal and human gene therapy studies. Adenoviral vectors exhibit several merits that make them suitable for liver regeneration gene therapy. Adenoviruses are highly hepatotropic and it is relatively easy to produce high titers of recombinant adenoviral particles\[57\]. Unlike retroviruses, adenoviruses transduce dividing and non-dividing cells and do not integrate into the host chromosomes, thereby eliminating the risk of insertional mutagenesis. These merits make adenoviral vectors suitable for proof of principle experimental studies to verify the effect of overexpression of a specific growth factor gene on liver regeneration. The major limitation of adenoviral vectors is their serious and potentially fatal toxicity as exemplified by the death of an 18-year-old man who received $6 \times 10^{11}$ viral particles/kg of E1/E4-deleted human adenovirus type 5 vector that contained human ornithine transcarbamylase cDNA\[58,59\]. Moreover, the severe immune response of the host contributes to the limited survival of the adenovirus.

### Table 1 General characteristics of most commonly used vectors

| System          | Size of insert (kb) | Infect non-dividing cell | Genomic integration | Duration of expression | Immune response |
|-----------------|---------------------|--------------------------|---------------------|------------------------|----------------|
| Adenovirus      |                     |                          | No                  | 3-4 wk                 | High           |
| 1st generation  | 5                   | Yes                      | No                  | Longer with            | High           |
| 2nd generation  | 8                   | Yes                      | No                  | Immunono-suppression   | Less           |
| Gutless         | 35                  | Yes                      | Yes and episomal    | Long-term              | Low            |
| Adeno-associated virus | < 4.8             | Yes                      | Yes, random         | Long-term              | Low            |
| Herpes simplex virus 1 | 35                | Yes                      | Yes, into active genes | Long-term              | Low            |
| Retrovirus      | ≤ 8                 | No                       | No                  | Short                  | Low            |
| Lentivirus      | ≤ 8                 | Yes                      | No                  | Short                  | Low            |
| Baculovirus     | > 20                | Yes                      | No                  | Short                  | Low            |
| Plasmid-naked   | Large               | Yes                      | No                  | Short                  | Low            |
| Plasmid-polymer | Large               | Yes                      | No                  | Short                  | Low            |
| Plasmid-lipid (liposomes) | Large      | Yes                      | No                  | Short                  | Low            |
DNA in targeted cells and results in transient expression of the therapeutic gene. Until resolved, adenoviral-vector-induced toxicity will limit its application in clinical gene therapy studies. The transient nature of gene expression with adenoviral vectors may be advantageous because the process of liver regeneration is usually completed in approximately 1 wk. However, liver regeneration is seldom the only goal of therapy. Treating associated liver fibrosis or cirrhosis requires a longer period of gene expression. Furthermore, transduction efficiency of diseased liver is much lower than that of healthy liver. Garcia-Bañuelos et al. have demonstrated that adenovirus-mediated gene transfer via the iliac vein at 3 \times 10^7 viral particles per rat resulted in approximate 40% transduction in livers made cirrhotic by chronic intoxication with carbon tetrachloride, compared with approximate 80% in control non-cirrhotic livers. In rats made cirrhotic by bile-duct obstruction only 10% efficiency of transduction was observed. Yu et al. have shown that NPCs are transduced with greater frequency than hepatocytes at all adenoviral titers tested, both \textit{in vitro} and \textit{in vivo}. After liver injury, adenoviral transduction is reduced for the development of liver cell types compared with that for cells from normal livers (at all virus titers). Again, transduction efficiency remains greater in NPCs than in hepatocytes after liver injury.

Non-viral vectors can be divided into two categories: physical and chemical. Physical methods involve the introduction of plasmid DNA into cells using electroporation, ultrasound, or hydrodynamic delivery. Chemical methods use lipid or polymer carriers that complex with DNA to deliver the transgene into cells. Several non-viral vectors have been used for \textit{in vivo} liver gene therapy including various liposome preparations, protein-DNA conjugates, nanoparticles, and naked or complexed DNA. Expression is usually both transient and at low level because the DNA is not stable in cells. Despite these limitations, non-viral vectors offer many advantages including being simple to use, ease of production of large quantities, and absence of host immune response.

A major advance in the intravascular delivery of vectors followed the development of the hydrodynamic injection technique. The technique involves rapid tail vein injection of a large volume of the vector (around 10% of the body weight of a mouse or rat) in a short time period (5-7 s in mice and 15-20 s in rats). The hydrodynamic method results in dramatically higher hepatic transfection efficiency compared to conventional injection. Typically, 10%–15% of hepatocytes are transected in mouse liver following injection of 10 mg plasmid, but levels up to 40% have been reported. Liver enzymes are transiently elevated and liver histology shows minimal damage that resolves within a week, which is similar to the results obtained from intravascular delivery into liver vessels. It has been postulated that increased pressure in the inferior vena cava causes retrovenous blood flow from the central to the portal vein, and the resultant increased intrahepatic vascular pressure promotes massive endocytosis that generates intracellular water movement that facilitates gene entry. There are multiple lines of evidence that the species differences in the diameter of sinusoidal fenestrae are a critical determinant of transgene expression after adenoviral transfer. The small diameter of fenestrae in humans should be considered in any rational design of gene therapy studies. Hydrodynamic gene transfer is highly successful in rodents. The significantly lower efficacy in higher species may also partially be due to species differences in liver architecture. Intrinsically, factors in particular compliance (elasticity) of the liver are likely to be crucial in determining the degree of swelling for a given level of intrahepatic vascular pressure. Liver compliance is likely to be the major reason for the low level of hydrodynamic gene delivery in the pig model, and will influence the effectiveness of the approach in humans, both in general and in different disease states.

This procedure has great limitations for application to clinical practice, therefore, a clinically relevant method for regional hydrodynamic delivery of vectors has been developed. The method entails the use of an occlusion balloon catheter into the inferior vena cava and retro dynamically injecting towards the liver and through the hepatic vein, 100 mL of the plasmid in saline solution (20 mg/mL), at a rate of 7.5 mL/s. This retrodynamically hepatic vein gene delivery method has been performed in pigs, and was as well tolerated as in mice and led to liver transgene expression, however, the plasma levels of the transgene protein were four orders of magnitude lower than those reached in the murine model. A variety of different modifications have been reported recently.

Recently, retrograde administration of adenoviruses into the common bile duct has been shown to induce efficient transgene expression in the liver without causing severe adverse effects, thus supporting the feasibility of adenovirus-mediated gene transfer into the liver in clinical settings by means of endoscopic retrograde cholangiography. Repeat administration of adenoviruses into the common bile duct is successful in re-expressing the transgene in the liver. This contrasts with the failure of re-expression of transgene following intravenous readministration of an adenoviral vector long after the initial administration.

### OVERVIEW OF PUBLISHED STUDIES

The general features of the reviewed gene therapy studies for enhancing liver regeneration are summarized in Table 2. Gene therapy investigations that fulfilled the following criteria were selected for review: (1) demonstrated, objectively, enhanced liver cell proliferation and or increased survival as compared with controls; (2) animals and/or livers receiving gene therapy were not genetically modified as they do not directly represent human liver diseases (e.g. liver cirrhosis, fibrosis or failure) in which liver regeneration has a critical role in recovery; and (3) gene therapy was administered \textit{in vivo}. The selection of homogeneous cohort studies based on these criteria allows us to delineate the main characteristics of these studies, and more importantly, envision what needs to be done in fu-
tured studies as a preparation for clinical trials. An overview of the different elements of gene therapy for liver regeneration studies are given below.

**Vector type**

Given the merits of adenoviruses as a powerful vector that has the highest transduction rate for liver cells, it is not surprising that two-thirds of all reviewed studies used it to prove the effect of the therapeutic gene (Table 2). It was the only viral vector used. The non-viral vectors employed in the rest of the reviewed studies are divided between naked DNA and liposomes. Despite the lower transfection rate of the non-viral vectors, their safety makes them suitable candidates for preclinical studies.

**Vector dose**

The administered adenoviral dose ranged between $1 \times 10^6$ pfu and $4 \times 10^7$ pfu with a dose of $1 \times 10^7$ pfu used in 80% of the studies. The average vector dose for mice was no different from that for rats despite considerable differences in their body weights. Phaneuf et al. have examined the effect on liver regeneration of increasing doses ($1 \times 10^6$ to $4 \times 10^7$ pfu) of adenoviral vector encoding for human HGF. They have found that DNA synthesis of hepatocytes and liver weight increased in a dose-dependent fashion, such that the maximal effect was seen after the infusion of $3 \times 10^7$ pfu, which resulted at day 5 in a $>130\%$ increase in relative liver mass, with little cytotoxic effect. The average single dose of naked DNA was 10-50 µg and that of liposomes was 50 nmol.

**Therapeutic genes**

By far the most studied therapeutic gene was HGF, which is not surprising given the fact that it is the single most important growth factor implicated in liver regeneration. It has been used in about two-thirds of studies, either alone or in combination with other factors such as VEGF, which has been used in a minority of studies. The combination of HGF and VEGF is one of the promising approaches for liver regeneration. The combination of HGF and VEGF can be useful in clinical trials due to the synergistic effects of these two factors on liver regeneration.

---

Table 2: Main features of reported gene therapy experiments.

| Vector, Ref. | Dose | Transgene (promoter) | Vector type | Measured parameters |
|-------------|------|----------------------|-------------|---------------------|
| Adenovirus vector |
| Hogaboam et al., 1999 | $1 \times 10^7$ pfu | r-MIP-2 | Liver injury, mice, IV | DNA synthesis, survival |
| Phaneuf et al., 2001 | $1 \times 10^7$ pfu | h-HGF (CMV) | Healthy, mice, IV | DNA synthesis, apoptosis and ALT |
| Shiozawa et al., 2000 | $1 \times 10^7$ pfu | r-HGF (CAG) | AAF/70% PH, rats, IV | Oval cell proliferation |
| Nomoto et al., 2000 | $1 \times 10^7$ pfu | r-HGF (CAG) | D-Gal/LPS liver failure, rats, IP | Apoptosis, survival |
| Hecht et al., 2004 | $1 \times 10^7$ TU | h-HIL-6 (CMV) | D-Gal liver failure, mice, IP | Survival, proliferation |
| Hwang et al., 2003 | $1 \times 10^7$ pfu | h-HGF (CAG) | TAA liver failure, mice, IV | Survival, DNA synthesis, no hepatic necrosis |
| Iwaki et al., 2003 | $2 \times 10^7$ pfu | m-MIF antisense | BCG-LPS liver failure, mice, IV | Survival |
| Oe et al., 2004 | $7 \times 10^7$ pfu | h-VEGF + or r-HGF (CAG) | AAF/70% PH, rats, IV | Oval cell proliferation, regeneration |
| Oe et al., 2005 | $7 \times 10^7$ pfu | h-VEGF, or h-VEGF (CAG) | DMN cirrhosis 70% PH, rats, PV | Survival, proliferation, SECs and hepatocytes proliferation |
| Wullaert et al., 2005 | $5 \times 10^7$ pfu | m-ABIN-1 (CMV) | TNF + Gal-liver injury, mice, IV | Survival, apoptosis, proliferaton |
| Ichiba et al., 2005 | $1 \times 10^7$ pfu | r-TPO (CAG) | AAF/70% PH, rats, IV | Survival, proliferation |
| Kishi et al., 2006 | $1 \times 10^7$ pfu | h-HB-EGF or h-HGF (RSV) | Fas-induced injury, mice, IV | Apoptosis and proliferation by both |
| Ozawa et al., 2006 | $5 \times 10^7$ pfu each | r-HGF, / or h-TGFβR2 (CAG) | DMN cirrhosis 10% PH, rats, PV | Proportion, survival, cirrhosis |
| Tan et al., 2006 | $1 \times 10^7$ pfu | m-HNF6 (CMV) | 70% PH, mice, IV | Proportion |
| Yusa et al., 2007 | $1 \times 10^7$ pfu | r-HGF, (CBA) | 85% PH, rats, IV | Apoptosis, proliferation, survival |
| Ueno et al., 2007 | $5 \times 10^7$ pfu | r-HGF (CAG) | DMN cirrhosis 70% PH, rats, sPV | Proportion, survival, cirrhosis, hepatitis |
| Atta et al., 2009 | $7 \times 10^7$ pfu | h-HGF, h-VEGF (CMV) | Healthy, dogs, IV | SEC and hepatocytes proliferation |
| Naked plasmid DNA |
| Yang et al., 2001 | $10-40$ µg/wk × 8 | h-HGF (CMV) | Healthy, mice, IV | Proliferation |
| Xue et al., 2003 | $50 \times 3$ | r-HGF | CCI liver injury 70% PH, mice, IM + | Proliferation |
| Zhang et al., 2005 | $200$ µg/kg per 12 h × 4 | r-ALR | CCI liver injury, rats, IV | ALT and AST, proliferation, survival |
| Horiguchi et al., 2006 | - | h-HGF | DMN cirrhosis, dogs, IA | ALT and AST, fibrosis, survival |
| HVJ Liposomes |
| Ueki et al., 1999 | $20$ or $40$ mg weekly × 4 | h-HGF (SRa) | DMN cirrhosis, rats, IM | Apoptosis, survival, 1r-HGF, fibrosis |
| Ogushi et al., 2003 | $50$ nmol | NF-kB decoy ODN | P. acnes-LPS liver injury, mice, PV | Survival, proliferation, apoptosis |
| Nishino et al., 2008 | $20$ µg | h-HGF (FrA) | DMN cirrhosis 70% PH, rats, PV | Survival, proliferation, apoptosis |
| Takahashi et al., 2009 | $50$ nmol | NF-kB decoy ODN | 90% PH, mice, PV | Survival, proliferation |

AAV: Acetylsalicylic acid; ALT: Alanine transaminase; AST: Aspartate transaminase; AABIN-1: A20 binding inhibitor of nuclear factor κB; ALR: Augmenter of liver regeneration; BCG: Bacille Calmette-Guérin; CAG: Chicken β-actin promoter and cytomegalovirus enhancer; CBA: Chicken β-actin; D-Gal: D-galactosamine; TNF: Tumor necrosis factor; HVJ: Hemagglutinating virus of Japan; DMN: Dimethylnitrosamine; EP: Electroporation; Gal: Galactosamine; h: Human; h-HIL-6: Human hyper-interleukin-6 (IL-6)-cDNA gene coding the human sIL-6R (amino acid residues 1-323) and human IL-6 (amino acid residues 29-212) fused by a synthetic DNA linker; HNF6: Hepatocyte nuclear factor 6; IA: Intra-arterial injection (hepatic artery); IM: Intramuscular injection; IP: Intraperitoneal injection; IV: Intravenous injection; M: Murine; HGF: Hepatocyte growth factor; MIF: Macrophage migration inhibitory factor; MIP-2: Macrophage inflammatory protein-2; VEGF: Vascular endothelial growth factor; CMV: Cytomegalovirus; ODN: Oligodeoxynucleotides; PH: Partial hepatectomy; LPS: Lipopolysaccharide; SECs: Sinusoidal endothelial cells; P. acnes: Propionibacterium acnes; PV: Portal vein injection; r: Rat; sPV: Selective portal vein injection; SFlu: Simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat; TAA: Thioacetamide; TGFβR2: Truncated transforming growth factor β type 2 receptor; TPO: Thrombopoietin; TU: Transducing units (1 pfu = 25 TU); vp: Viral particles (1 pfu = 100 pfu).
or in combination with other growth factors (Table 2). Other genetic materials used include growth factors, cytokines or transcription factors involved in direct liver cell proliferation, e.g. VEGF, HB-EGF, C/EBPβ, and IL-6. Two studies have reported the use of antisense ODN to NF-κB (NF-κB decoy ODN) encapsulated in hemagglutinating virus of Japan (HVJ) liposomes to prevent endotoxin- or massive hepatectomy-induced liver failure [50,52]. Antisense ODNs are not natural genes, and they are short (15-20 bases in length) synthetic oligonucleotides that are designed to hybridize to RNA through Watson-Crick base pairing. Upon binding to the target RNA, ODNs prevent expression of the encoded gene product. Although stimulation of the transcription factor NF-κB in Kupffer cells, with production of inflammatory cytokines, has been shown to be involved in liver proliferation, excessive production of cytokines is thought to be responsible for liver failure following excessive hepatectomy [53].

**Route of administration**

The liver is an attractive target for *in vivo* gene transfer studies because hepatocytes are readily accessible *via* the blood stream. The endothelium of hepatic sinusoids displays fenestrations that are 100 nm wide and that allow macromolecules such as viral particles to cross the endothelium and reach hepatocytes. Moreover, the hepatic blood flow represents one-fifth of the cardiac output. Thus, any particle injected into the blood circulation can quickly reach the liver [34]. For this reason, the vascular route constitutes the most commonly used in 80% of the reviewed studies. The intravenous route is the commonest among the vascular routes not only because it is the easiest route compared with intra-arterial or portal vein administration, but also due to the enhanced transduction rate following the recent modification of the hydrodynamic technique mentioned above.

**Duration of transgene expression**

Few of the reviewed studies have reported the duration of expression of the transduced gene or its protein [39,45,81,83,85,87,88,93,95]. Those studies that had extended observation periods have shown that the duration of transgene expression does not extend beyond 1 wk following vector administration [39,81,83,87,88,94]. These data agree with the accumulated knowledge that gene therapy using adenoviral vectors or non-viral naked DNA and liposomes confers a limited duration of gene expression. Moreover, it should be noted that the efficiency of gene transduction, which directly affects the duration of gene expression, is lower in cirrhotic liver than in normal liver due to capillarization of sinusoidal endothelial cells as a result of the decreased size or loss of the fenestrae of sinusoidal endothelial cells [99]. Nishino et al [87] have demonstrated that only 5%-6% of hepatocytes in cirrhotic rat livers were successfully transfected with human HGF plasmid enveloped in HVJ liposomes.

**Non-hepatic gene transfection**

There was a tendency towards excluding gene therapy studies for liver regeneration in which gene transduction involved organs other than the liver, e.g. skeletal muscles. Although this could be appropriate for the sake of presenting a homogeneous group of investigations, it was felt however that this would have omitted an important cluster of studies that represented an emerging direction in gene therapy for liver regeneration. In this regard, two studies used liposomes and naked plasmid to transduce skeletal muscles with HGF in animals with liver cirrhosis. They demonstrated expression of the transduced HGF gene and elevation of its plasma levels that exerted proliferative and antifibrotic effects on the liver [90,92].

**FUTURE PERSPECTIVES**

In 20 years of gene therapy research, there have been few studies that have aimed at enhancing liver regeneration. However, the accumulated knowledge from these studies has allowed the validation of proof of principle gene therapy investigations for promoting liver regeneration in different animal models of liver diseases. Future progress in this field is expected to tackle several points.

First, determination of the combination of gene therapy that works better for a specific disease condition. As mentioned above, enhancing liver regeneration is seldom the only goal of therapy. Treating associated liver fibrosis/cirrhosis or toxic injury requires the combined effects of genetic materials such as growth factor genes and antisense ODN. This should be based on the outcomes drawn from experimental comparative studies of different combinations of therapeutic genes for each defined disease. An example of such comparative studies is that of Ozawa et al [103]. In rats with liver cirrhosis, combination gene therapy of HGF, a powerful liver mitogen, and truncated type II TGF-β receptor that specifically inhibits TGF-β signaling that is responsible for progression of liver fibrosis [109], resulted in decreased liver fibrosis and improved liver function, compared with monotherapy with either gene alone. These studies provide an opportunity to shed light on how the administered genes influence the pathogenesis of the multifactorial disease process. Also, it could identify synergistic combinations that could enhance regeneration, disease resolution and reduce the amount of transferred genetic material. An example of such studies would make use of HGF and NF-κB decoy ODN, which prevents excessive cytokine production, to prevent hepatocyte apoptosis and enhance regeneration after massive resection or liver injury [93].

Secondly, evaluation of the trade-off of risk against the benefits of viral vs non-viral gene therapy. Unlike gene therapy for liver genetic diseases that require a high rate of liver transduction to express the therapeutic protein efficiently in the systemic circulation, at a clinically relevant concentration, gene therapy for liver regeneration or resolution of fibrosis aims at locally expressing the desired proteins, which act in an autocrine or paracrine fashion [98]. Thus, despite non-viral systems having a lower transfection rate, they are safer, easy to produce in large quantities, and can be repeatedly administered, which can
aid in gauging the amount and duration of gene expression. Moreover, hydrodynamic injection in murine models and its clinically relevant retrodextric hepatic vein gene delivery in large animals have dramatically increased transfection efficiency of non-viral systems.

Thirdly, employing the recently developed vectors that target specific liver cell types, and promoters that are capable of liver-specific sustained transgene expression in gene therapy studies to augment liver regeneration and treat associated liver injury; These new developments can be summarized as follows: (1) Cell-specific expression of therapeutic genes of interest is an extremely attractive strategy in gene therapy. Several investigators have developed selective hepatic cell delivery systems using receptors that are unique to and highly expressed by different liver cell types: (A) The asialoglycoprotein receptor (ASGPR) on the hepatocyte membrane is a specific targeting marker for gene and drug delivery. Studies have targeted the hepatocyte ASGPR using its natural ligand, asialoorosomucoid\cite{105,106,107}. Chiba et al\cite{106} recently have developed cationically modified biocompatible phospholipid polymer conjugated with hepatitis B surface antigen for the specific transfer of genes into human hepatocytes; (B) Quiescent HSCs lack specific receptors or motifs on their cell surface, thus, attempts to target HSCs have been a challenging task\cite{108}. (a) The mannose 6-phosphate/insulin-like growth factor II (M6P/IGF-II) receptor expression is increased on activated HSCs, particularly during fibrosis. The receptor has binding sites for IGF-II and M6P-containing ligands\cite{109}. Beljaars et al\cite{109} have developed a carrier system that consists of human serum albumin modified with M6P, which binds to the M6P/IGF-II receptors on HSCs\cite{104,109,110}. (b) Vitamin A receptors on HSCs have been used to deliver siRNA against collagen-specific chaperone heat shock protein 47 via vitamin A-coupled liposomes\cite{111,112}; and (c) Liposomes labeled with a cyclic RGD-peptide that recognizes the collagen type VI receptors\cite{113,114,115}; (C) Sinusoidal endothelial cells (SECs) possess unique hyaluronic receptors that recognize and internalize hyaluronic acid (HA). SECs have been targeted using HA, the endogenous ligand for the HA receptor for endocytosis\cite{111,112,116}; and (D) Kupffer cells possess receptors that recognize galactose and N-acetylgalactosamine. Studies have shown that galactosylation can target various DNA preparations including liposomes, low-density lipoprotein and chitosan polymer to Kupffer cells\cite{113,114,115}; and (2) Liver-specific sustained transgene expression can be obtained at very high levels from optimized promoters\cite{116}. Many experimental gene therapy vectors described in this review express transgenes under the control of non-specific promoters such as CMV, Rous sarcoma virus, simian virus 40 (SV40) and mammalian elongation factor 1α (EF1α) (Table 2). These promoters direct strong gene expression but are shut off rapidly in vivo\cite{117,118}. A tissue-specific promoter is a promoter that has activity in only certain cell types. Use of a tissue-specific promoter in the expression cassette can restrict unwanted transgene expression as well as facilitate persistent transgene expression\cite{119}. Ongoing developments are based on two liver-specific promoters, the albumin promoter and the α1 antitrypsin promoter. Wooddell et al\cite{120} have demonstrated that when using a plasmid vector that contains albumin promoter combined with an α-fetoprotein (AFP) MER II enhancer, 5′ intron from the factor IX gene, and the 3′UTR from the albumin gene, including intron 14, the reporter gene expression levels remained high for 1 year, at levels comparable to those obtained from the CMV promoter on day 1. Ziegler et al\cite{121} have shown that intravenous administration of a recombinant AAV2 vector encoding human α-galactosidase A under the transcriptional control of a liver-restricted enhancer/promoter consisted of human serum albumin promoter (nucleotides -486 to +20), to which were appended two copies of the human prothrombin enhancer (nucleotides -940 to -860). The enhancers were placed 5′ of the promoter in the forward orientation. This vector mediated sustained hepatic expression of α-galactosidase A for 12 mo and was associated with a significantly reduced immune response to the expressed enzyme. Several investigators have reported encouraging long expression of transgenes using different modifications of α1 antitrypsin promoter\cite{122,123}. Jacobs and his colleagues have compared 22 hepatocyte-specific expression cassettes and have found that a promoter that consists of an 890-bp human α1-antitrypsin promoter and two copies of the 160-bp α1-microglobulin enhancer results in the highest expression levels\cite{124}. Comparisons between different liver-specific promoters have shown that α1-antitrypsin promoters induce higher levels and prolonged expression of transgenes than other liver-specific promoters such as AFP and albumin promoter\cite{125,126}. The most recent investigations have shown the unlimited possibilities for gene therapy modifications. Li et al\cite{127} have developed a small DNA fragment (347 bp) from the AAV chromosome 19 integration site that is capable of providing efficient and enhanced liver-specific transcription when used in recombinant AAV vectors. Previously described tissue-specific promoters for gene therapy are typically too big for AAV vectors. Wolff et al\cite{128}, in an effort to increase long-term expression of transgene products, have designed a plasmid DNA vector under the control of a tissue-specific promoter and have included microRNA target sites in the transcripts, in order to silence expression in antigen-presenting cells.

**CONCLUSION**

The success of several proof of principle studies of gene therapy for liver regeneration, coupled with the recent extensive search for the mechanisms of selective targeting of specific liver cells, should pave the way towards future clinical trials. As liver regeneration is usually an integral part of the therapeutic goals of many liver diseases, gene therapy to enhance liver regeneration needs to be combined with gene therapy for associated liver disease. Consequently, clinically relevant gene transfer protocols should be developed to address specific goals of such combined gene therapy trials.
REFERENCES

1. Tian Y, Jochum W, Georgiev P, Moritz W, Graf R, Clavien PA. Kupffer cell-dependent TNF-alpha signaling mediates injury to the arterialized small-for-size liver transplantation in the mouse. Proc Natl Acad Sci USA 2006; 103: 4598-4603
2. Inoue H, Yokoyama F, Kita Y, Yoshii H, Tsujimoto T, Deguchi A, Nakai S, Morishita A, Uchida N, Masaki T, Watanabe S, Kuriyama S. Relationship between the proliferative capability of hepatocytes and the intrahepatic expression of hepatocyte growth factor and c-Met in the course of cirrhosis development in rats. Int J Mol Med 2006; 17: 857-864
3. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology 2006; 43: S45-S53
4. Michalopoulos GK. Liver regeneration. J Cell Physiol 2007; 213: 286-300
5. Kaido T, Yamaoka S, Seto S, Funaki N, Kasamatsu T, Tanaka J, Nakamura T, Imamura M. Continuous hepatocyte growth factor supply prevents lipopolysaccharide-induced liver injury in rats. FEBS Lett 1997; 411: 378-382
6. Kaido T, Yoshikawa A, Seto S, Yamaoka S, Sato M, Ishii T, Inoue K, Imamura M. Hepatocyte growth factor supply accelerates compensatory hypertrophy caused by portal branch ligation in normal and jaundiced rats. J Surg Res 1999; 85: 115-119
7. Kaido T, Yoshikawa A, Seto S, Yamaoka S, Sato M, Ishii T, Imamura M. Portal branch ligation with a continuous hepatocyte growth factor supply makes extensive hepatocyte possible in cirrhotic rats. Hepatology 1998; 28: 756-760
8. Yoshikawa A, Kaido T, Seto S, Yamaoka S, Sato M, Ishii T, Imamura M. Hepatocyte growth factor promotes liver regeneration with prompt improvement of hyperbilirubinemia in hepatoma thresholded cholestatic rats. J Surg Res 1998; 78: 54-59
9. Namisaki T, Yoshii H, Kuriyama S, Kojima H, Yoshii J, Ike-naka Y, Noguchi R, Sakurai S, Yanase K, Kitade M, Yamazaki M, Asada K, Tsujimoto T, Akahane T, Uemura M, Fukui H. A potent angiogenic factor, vascular endothelial growth factor, improves the survival of the on-going acute hepatic failure in rats. Hepatol Res 2006; 35: 199-203
10. Michalopoulos GK, DeFrances MC. Liver regeneration. Science 1997; 276: 60-66
11. Fausto N. Liver regeneration. J Hepatol 2000; 32: 19-31
12. Overturf K, al-Dhalimy M, Ou CN, Finegold M, Grompe M. Serial transplantation reveals the stem-cell-like regenerative potential of adult mouse hepatocytes. Am J Pathol 1997; 151: 1273-1280
13. Rhim JA, Sandgren EP, Degen JL, Palmer RD, Brinster RL. Replacement of diseased mouse liver by hepatic cell transplantation. Science 1994; 263: 1149-1152
14. Rhim JA, Sandgren EP, Palmer RD, Brinster RL. Complete reconstitution of mouse liver with xenogenic hepatocytes. Proc Natl Acad Sci USA 1995; 92: 4942-4946
15. Sandgren EP, Palmer RD, Heckel JL, Daugherty CC, Brinster RL, Degen JL. Complete hepatic regeneration after somatic deletion of an albumin-plasminogen activator transgene. Cell 1999; 91: 245-256
16. Albrecht JH, Hansen LK. Cyclin D1 promotes mitogen-independent cell cycle progression in hepatocytes. Cell Growth Differ 1999; 10: 397-404
17. Fausto N, Laird AD, Webber EM. Liver regeneration. 2. Role of growth factors and cytokines in hepatic regeneration. J Biol Chem 2000; 275: 7093-7100
18. Shioti G, Kunisada T, Oyama K, Udagawa A, Nomi T, Tanaka K, Tsutsui A, Isono M, Nakamura T, Hamada H, Sakatani T, Sell S, Sato K, Ito H, Kawasaki H. In vivo transfer of hepatocyte growth factor gene accelerates proliferation of hepatic oval cells in a 2-acetylaminofluorene/partial hepatectomy model in rats. J Hepatol 2000; 32: 835-844
19. Fausto N. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. Hepatology 2004; 39: 1477-1487
20. Kobayashi Y, Hamaoue M, Ueno S, Aikou T, Tanabe G,
Mitsue S, Matsumoto K, Nakamura T. Induction of hepatocyte growth by intraportal infusion of HGF into beagle dogs. Biochim Biophys Acta 1996; 220: 7-12

43 Shiota G, Wang TC, Nakamura T, Schmidt EV. Hepatocyte growth factor in transgenic mice: effects on hepatocyte growth, liver regeneration and gene expression. Hepatology 1994; 19: 962-972

44 Kahi NC, Takahashi T, Ushikoshi H, Nagano S, Yuge K, Esaki M, Kawai T, Goto K, Murofushi Y, Fujisawa T, Fujiwara H, Kosai K. In vivo hepatic HB-EFG gene transduction inhibits Fas-induced liver injury and induces liver regeneration in mice: a comparative study to HGF. J Hepatol 2006; 44: 1046-1054

45 Oe H, Kaido T, Furuyama H, Mori A, Imamura M. Simultaneous transfer of vascular endothelial growth factor and hepatocyte growth factor genes effectively promotes liver regeneration after hepatectomy in cirrhotic rats. Hepatogastroenterology 2004; 51: 1641-1647

46 Planeuf D, Chen SJ, Wilson JM. Intravenous injection of an adenovirus encoding hepatocyte growth factor results in liver growth and has a protective effect against apoptosis. Mol Med 2000; 6: 96-103

47 Ozawa S, Uchiyama K, Nakamori M, Ueda K, Iwashashi M, Ueno H, Muragaki Y, Ooshima A, Yamae H. Combination gene therapy of HGF and truncated type II TGF-beta receptor gene induces liver regeneration and allows for efficient retrovirus-mediated gene transduction of hepatocytes in vivo. AAPS J 2003; 5: 683-687

48 Iwaki T, Sugimura M, Nishihira J, Matsurua T, Kobayashi T, Kanayama N. Recombinant adenovirus vector bearing antisense macrophage migration inhibitory factor cDNA prevents acute lipopolysaccharide-induced liver failure in mice. Lab Invest 2003; 83: 561-570

49 Takahashi T, Togo S, Kunimoto T, Watanabe K, Kubota T, Ichikawa Y, Endo I, Kunisaki C, Nagashima Y, Fujimoto J, Shimada H. Transfection of NF-kappB decoy oligodeoxynucleotides into macrophages reduces murine fatal liver failure after excessive hepatectomy. J Surg Res 2009; 154: 179-186

50 Prieto J, Qian C, Sangro B, Meler J, Mazzolini G. Biologic therapy of liver tumors. Surg Clin North Am 2004; 84: 673-696

51 Jin X, Yang YD, Li YM. Gene therapy: regulations, ethics and its practicalities in liver disease. World J Gastroenterol 2008; 14: 361-371

52 Kren BT, Chowdhury NR, Chowdhury JR, Steer CJ. Gene therapy as an alternative to liver transplantation. Liver Transpl 2002; 8: 1089-1108

53 Nguyen TH, Ferry N. Liver gene therapy: advances and hurdles. Gene Ther 2004; 11 Suppl 1: S76-S84

54 Lieber A, Vranken Peeters MJ, Meuse L, Fausto N, Perkins J, Kay MA. Adenovirus-mediated urokinase gene transfer induces liver regeneration and allows for efficient retrovirus transduction of hepatocytes in vivo. Proc Natl Acad Sci USA 1995; 92: 6210-6214

55 Patijn GA, Lieber A, Schowalter DB, Schwall R, Kay MA. Hepatocyte growth factor induces hepatocyte proliferation in vivo and allows for efficient retroviral-mediated gene transfer in mice. Hepatology 1998; 28: 707-716

56 Xia D, Zhang MM, Yan LN. Recent advances in liver-directed gene transfer vectors. Hepatobiliary Pancreatic Dis Int 2004; 3: 322-326

57 Raper SE, Chirumule N, Lee FS, Wivel NA, Bagg A, Gao GP, Wilson JM, Batshaw ML. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. Mol Genet Metab 2003; 80: 148-158

58 Raper SE. Gene therapy: the good, the bad, and the ugly. Surgery 2005; 137: 487-492

59 Garcia-Banuelos J, Siller-Lopez F, Miranda A, Aguilar LK, Aguilar-Cordova E, Armendariz-Borunda J. Cirrhotic rat livers with extensive fibrosis can be safely transduced with clinical-grade adenoviral vectors. Evidence of cirrhosis reversal. Gene Ther 2002; 9: 127-134

60 Yu Q, Que LG, Rockey DC. Adenovirus-mediated gene transfer to nonparenchymal cells in normal and injured liver. Am J Physiol Gastrointest Liver Physiol 2002; 282: C565-C572

61 Gao X, Kim KS, Liu D. Nonviral gene delivery: what we know and what is next. AAPS J 2007; 9: E92-E104

62 Nguyen AT, Dow AC, Kupiec-Weglinski J, Busuttil RW, Lipshutz GS. Evaluation of gene promoters for liver expression by hydrodynamic gene transfer. J Surg Res 2008; 148: 60-66

63 Kren BT, Bandyopadhyay P, Chowdhury NR, Chowdhury JR, Steer CJ. Oligonucleotide-mediated site-directed gene repair. Methods Enzymol 2002; 346: 14-35

64 Pathak A, Vyas SP, Gupta KC. Nano-vectors for efficient liver specific gene transfer. Int J Nanomedicine 2008; 3: 31-49

65 Brunetti-Pierri N, Lee B. Gene therapy for inborn errors of liver metabolism. Mol Genet Metab 2008; 95: 13-24

66 Herweijer H, Wolff JA. Progress and prospects: naked DNA gene transfer and therapy. Gene Ther 2003; 10: 453-458

67 Crespo A, Peydro A, Dafi F, Benet M, Calvette J, Revert F, Alleno SF. Hydrodynamic liver gene transfer mechanism involves transient sinusoidal blood stasis and massive hepatocyte endocytic vesicles. Gene Ther 2005; 12: 927-935

68 Arad U, Zeira E, El-Latif MA, Mukherjee S, Mitchell L, Pappo O, Galun E, Oppenheim A. Liver-targeted gene therapy by SV40-based vectors using the hydrodynamic injection method. Hum Gene Ther 2005; 16: 361-371

69 Jacobs F, Feng Y, Van Craeyveld L, Lievens J, Snoeys J, De Geest B. Species differences in hepatocyte-directed gene transfer: implications for clinical translation. Curr Gene Ther 2009; 9: 83-90

70 Sawyer GJ, Rela M, Davenport M, Whitehorne M, Zhang X, Fabre JW. Hydrodynamic gene delivery to the liver: theoretical and practical issues for clinical application. Curr Gene Ther 2009; 9: 128-135

71 Eastman SJ, Baskin KM, Hodges BL, Chu Q, Gates A, Dreusickie R, Anderson S, Scheule RK. Development of catheter-based procedures for transducing the isolated rabbit liver with plasmid DNA. Hum Gene Ther 2002; 13: 2065-2077

72 Brunetti-Pierri N, Stapleton GE, Palmer DJ, Zuo Y, Mane VP, Finegold MJ, Beaudet AL, Leland MM, Mullins CE, Ng P. Pseudo-hydrodynamic delivery of helper-dependent adenoviral vectors into non-human primates for liver-directed gene therapy. Mol Ther 2007; 15: 752-760

73 Dariel A, Nguyen TH, Pichard V, Schmitt F, Aubert D, Ferry N, Podevin G. A new surgical approach to improve gene transfer in liver using lentiviral vectors. J Pediatr Surg 2009; 44: 517-522

74 Kuriyama S, Yoshii H, Tominaga K, Tsujinoue H, Nakatani T, Tsujimoto T, Okuda H, Akahane T, Deguchi A, Nakai S, Masaki T, Uchida N. Repetitive and safe transgene expression. J Gene Med 2005; 7: 148-158

75 Kuriyama S, Yoshii H, Deguchi A, Nakai S, Ogawa M, Nonomura T, Kimura Y, Inoue H, Kinekawa F, Tsujimoto M, Masaki T, Kurokokohchi K, Uchida N. Safe and efficient transgene expression in rat hepatocytes induced by adenoviral administration into the biliary tract. Oncol Rep 2005; 13: 825-830

76 Peeters MJ, Patijn GA, Lieber A, Meuse L, Kay MA. Adenovirus-mediated hepatic gene transfer in mice: comparison of intravascular and biliary administration. Hum Gene Ther 1996; 7: 1693-1699

77 Tominaga K, Kuriyama S, Yoshii H, Deguchi A, Kita Y, Funakoshi F, Masaki T, Kurokokohchi K, Uchida N, Tsujimoto T, Fukui H. Repeated adenoviral administration into the biliary tract can induce repeated expression of the original gene construct in rat livers without immunosuppressive strategies. Gastro 2004; 53: 1167-1173

78 Tsujinoue H, Kuriyama S, Tominaga K, Okuda H, Nakatani T, Yoshii H, Tsujimoto T, Akahane T, Asada K, Fukui H...
Intravenous readministration of an adenoviral vector performed long after the initial administration failed to induce re-expression of the original transgene in rats. *Int J Oncol* 2001; 18: 575-580

Hogaboam CM, Simpson KJ, Chensue SW, Steinhauser ML, Lukacs NW, Gauldie J, Strieter RM, Kunkel SL. Macrophage inflammatory protein-2 gene therapy attenuates adenosvir- and acetaminophen-mediated hepatic injury. *Gene Ther* 1999; 6: 573-584

Hwang TH, Yoon BC, Jeong JS, Seo SY, Lee HJ. A single administration of adenoviral-mediated HGF cDNA permits survival of mice from acute hepatic failure. *Life Sci* 2003; 72: 851-861

Oe H, Kaido T, Mori A, Onodera H, Imamura M. Hepatocyte growth factor as well as vascular endothelial growth factor gene induction effectively promotes liver regeneration after hepatectomy in Solt-Farber rats. *Hepatogastroenterology* 2005; 52: 1393-1397

Ueno M, Uchiyama K, Nakamori M, Ueda K, Iwahashi M, Ozawa S, Yamaue H. Adenoviral vector expressing hepatocyte growth factor promotes liver regeneration by preoperative injection: the advantages of performing selective injection to the remnant lobe. *Surgery* 2007; 141: 511-519

Wullaert A, Wielocks B, Van Hulvel S, Bogaert V, De Geest B, Papeleu P, Schotte P, El Bakkouri K, Heyninck K, Libert C, Beyaert R. Adenoviral gene transfer of ABLIN-1 protects mice from TNF/galactosamine-induced acute liver failure and lethality. *Hepatology* 2005; 42: 381-389

Yasuo I, Tsukada K, Hirai R, Ota T, Murakami M, Naito M, Doiwa H, Date H, Shimizu N. Effects of adenoviral-mediat-ed hepatocyte growth factor on liver regeneration after massive hepatectomy in rats. *Acta Med Okayama* 2007; 61: 81-88

Horiguchi K, Hiran T, Ueki T, Hirakawa K, Fujimoto J. Treating liver cirrhosis in dogs with hepatocyte growth factor gene therapy via the hepatic artery. *J Hepatobiliary Pancreat Surg* 2009; 16: 171-177

Nishino M, Umiro Y, Ueki T, Hirano T, Fujimoto J. Hepatocyte growth factor improves survival after partial hepatectomy in cirrhotic rats suppressing apoptosis of hepatocytes. *Surgery* 2008; 144: 374-384

Ueki T, Kaneda Y, Tsutsui H, Nakanishi K, Sawaya Y, Morishita R, Matsumoto K, Nakamura T, Takahashi H, Okamoto E, Fujimoto J. Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Acta Med Okayama* 2007; 61: 81-88

Xue F, Takahara T, Yata Y, Kuwabara Y, Shinno E, Nonome K, Minemura M, Takahara S, Li X, Yamato E, Miura N, Tajima F, Kurimasa A, Hamada H, Shiota G. Dual effects of adenoviral-mediated hepatocyte growth factor in dogs enhances liver proliferation without systemic growth factor elevation. *Liver Int* 2009; 29: 1022-1030

Ichiba M, Shimomura T, Murai R, Hashiguchi K, Saeki T, Yoshida Y, Kanbe T, Tanabe N, Tsuchiya H, Miura N, Tajima F, Kurimasa A, Hamada H, Shiota G. Dual effects of adeno-virus-mediated thrombopoietin gene transfer on hepatic oval cell proliferation and platelet counts. *Biochem Biophys Res Commun* 2005; 335: 723-729

Nomi T, Shiota G, Isom T, Sato K, Kawasaki H. Adenosvir-mediated hepatocyte growth factor gene transfer prevents lethal liver failure in rats. *Biochem Biophys Res Commun* 2000; 278: 338-343

Tan Y, Yoshida Y, Hughes DE, Costa RH. Increased expression of hepatocyte nuclear factor 6 stimulates hepatocyte pro-liferation during mouse liver regeneration. *Gastroenterology* 2006; 130: 1283-1300

Isoda K, Koide H, Kojima M, Arita E, Ikakku M, Higashiya S, Tashiro F, Yamato E, Miyazaki J, Kawase M, Yagi K. Stimulation of hepatocyte survival and suppression of CCl4-induced liver injury by the adenovirally introduced C/EBP-beta gene. *Biochem Biophys Res Commun* 2005; 329: 182-187

Wang CH, Jawan B, Lee TH, Hung KS, Chou WY, Lu CN, Liu JK, Chen YJ. Single injection of naked plasmid encoding alpha-melanoctye-stimulating hormone protects against thioacetamide-induced acute liver failure in mice. *Biochem Biophys Res Commun* 2004; 322: 153-161

Xu B, Broome U, Uzunel M, Nava S, Ge X, Kumagai-Braesch M, Hulteny K, Christenson B, Ericzon BG, Holgersson J, Sumitran-Holgersson S. Capillarization of hepatic sinusoid by liver endothelial cell-reactive autoantibodies in patients with cirrhosis and chronic hepatitis. *Am J Pathol* 2003; 163: 1275-1289

Nakamura T, Sakata R, Ueno T, Sata M, Ueno H. Inhibition of transforming growth factor beta prevents progression of liver fibrosis and enhances hepatocyte regeneration in di-methylthiourea-treated rats. *Hepatology* 2000; 32: 247-255

Kren BT, Unger GM, Sjeklocha L, Trossen AA, Kornman V, Diethelm-Okitaka BM, Reding MT, Steer CJ. Nanocapsule-delivered Sleeping Beauty mediates therapeutic Factor VIII expression in liver sinusoidal endothelial cells of hemophilia A mice. *J Clin Invest* 2009; 119: 2086-2099

Zhang Y, Rong Qi X, Gao Y, Wei L, Maitani Y, Nagai T. Mechanisms of co-modified liver-targeting liposomes as gene delivery carriers based on cellular uptake and antigens inhibi-tion effect. *J Control Release* 2007; 117: 281-290

Chiba N, Ueda M, Shimada T, Jinno H, Watanabe J, Ishihara K, Kitajima M. Development of gene vectors for pinpoint target-geting to human hepatocytes by cationically modified poly-mer complexes. *Eur Surg Res* 2007; 39: 23-34

Beljaars L, Meijer DK, Poelstra K. Targeting hepatic stellate cells for cell-specific treatment of liver fibrosis. *Front Biosci* 2002; 7: e214-e222

Beljaars L, Molema G, Weert B, Bonnema H, Olinga P, Groothuis GM, Meijer DK, Poelstra K. Albumin modified with mannose 6-phosphate: A potential carrier for selective delivery of antifibrotic drugs to rat and human hepatic stel-late cells. *Hepatology* 1999; 29: 1486-1493

Gonzalo T, Beljaars L, van de Bovenkamp M, Temmink K, van Loenen AM, Reker-Smit C, Meijer DK, Lacombe M, Op-dam F, Kéri G, Orfi L, Poelstra K, Kok RJ. Local inhibition of liver fibrosis by specific delivery of a platelet-derived growth factor kinase inhibitor to hepatic stellate cells. *J Pharmacol Exp Ther* 2007; 321: 856-865

Greupink R, Bakker HI, Bouma W, Reker-Smit C, Meijer DK, Beljaars L, Poelstra K. The antiproliferative drug doxorubicin inhibits liver fibrosis in bile duct-ligated rats and can be selectively delivered to hepatic stellate cells in vivo. *J Pharmacol Exp Ther* 2006; 317: 514-521

Sato Y, Murase K, Kato J, Kobune M, Sato T, Kawano Y, Takimoto R, Takada K, Miyashita K, Matsuenga T, Takayama T, Niitsu Y. Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone. *Nat Biotechnol* 2008; 26: 431-442

Beljaars L, Molema G, Schuppan D, Geerts A, De Bleser PJ, Weert B, Meijer DK, Poelstra K. Successful targeting to rat hepatic stellate cells using albumin modified with cyclic pep-p
targeting delivery of antisense oligonucleotides into Kupffer cells protects rats from fulminant hepatitis.

Bijsterbosch MK, Manoharan M, Dorland R, Waarlo IH, Collen D, De Geest B. Direct comparison of adenoviral and non-viral hydrodynamic gene transfer. *Hum Gene Ther* 2008; 19: 1297-1305

Van Linthout S, Collen D, De Geest B. Effect of promoters and enhancers on expression, transgene DNA persistence, and hepatotoxicity after adenoviral gene transfer of human apolipoprotein A-1. *Hum Gene Ther* 2002; 13: 829-840

Jacobs F, Snoeys J, Feng Y, Van Craeyveld E, Lievens J, Armentano D, Cheng SH, De Geest B. Direct comparison of hepatocyte-specific expression cassettes following adeno- and nonviral hydrodynamic gene transfer. *Gene Ther* 2008; 15: 594-603

Al-Dosari M, Zhang G, Knapp JE, Liu D. Evaluation of viral and mammalian promoters for driving transgene expression in mouse liver. *Biotechnol Bioeng* 2006; 93: 673-678

Kramer MG, Barajas M, Razquín N, Berraondo P, Rodrigo M, Wu C, Qian C, Fortes P, Prieto J. In vitro and in vivo comparative study of chimeric liver-specific promoters. *Mol Ther* 2003; 7: 375-385

Lam PY, Sia KC, Khong JH, De Geest B, Lim KS, Ho IA, Wang GY, Miao LV, Huynh H, Hui KM. An efficient and safe herpes simplex virus type 1 ampiclon vector for transcriptionally targeted therapy of human hepatocellular carcinomas. *Mol Ther* 2007; 15: 1129-1136

Li C, Hirsch M, Carter P, Asokan A, Zhou X, Wu Z, Samulski RJ. A small regulatory element from chromosome 19 enhances liver-specific gene expression. *Gene Ther* 2009; 16: 43-51

Wolff LJ, Wolff JA, Sebestyén MG. Effect of tissue-specific promoters and microRNA recognition elements on stability of transgene expression after hydrodynamic naked plasmid DNA delivery. *Hum Gene Ther* 2009; 20: 374-388

S- Editor Tian L  L- Editor Kerr C  E- Editor Lin YP