

Gene therapy applications in gastroenterology and hepatology

Catherine H Wu PhD¹, Lanlan Shen MD PhD², George Y Wu MD PhD¹

CH Wu, L Shen, GY Wu. Gene therapy applications in gastroenterology and hepatology. *Can J Gastroenterol* 2000;14(1): 57-61. Advantages and disadvantages of viral vectors and nonviral vectors for gene delivery to digestive organs are reviewed. Advances in systems for the introduction of new gene expression are described, including self-deleting retroviral transfer vectors, chimeric viruses and chimeric oligonucleotides. Systems for inhibition of gene expression are discussed, including antisense oligonucleotides, ribozymes and dominant-negative genes.

Key Words: Antisense; Chimeric nucleotides; Gene therapy; Ribozymes; Vectors

Les applications de la thérapie génique en gastro-entérologie et en hépatologie

RÉSUMÉ : On passe ici en revue les avantages et les inconvénients des vecteurs viraux et non viraux pour l'acheminement de la thérapie génique aux organes de la digestion. On y décrit les progrès enregistrés au chapitre des systèmes mis au point pour l'introduction de nouvelles expressions géniques, y compris les vecteurs de transfert rétroviraux à auto-élimination, les virus chimériques et les oligonucléotides chimériques. Les systèmes mis au point pour l'inhibition de l'expression génique sont également abordés, notamment, les oligonucléotides antisens, les ribozymes et les gènes dominante négatifs.

VIRAL VECTORS

The most often frequently used viral vectors are retroviruses, adenoviruses and adeno-associated viruses (AAVs).

Retroviruses

Strategy for use of retroviruses as vectors: Replication-defective retroviral vectors, such as recombinant Moloney Murine virus, are the most popular delivery system. Replication-related viral genes are replaced with a gene of interest in a plasmid that contains a packaging signal, a sequence that is required for encapsidation. A packaging cell line is prepared by stable transfection with missing genes encoding essential replication-related viral genes. When the

plasmid containing the gene of interest and the packaging signal are introduced into a packaging cell line, only RNA containing the packaging signal is packaged into a viral particle. However, because the replication-related viral genes are missing, the new virus is replication-defective. When the defective retroviral particle binds to the target cell membrane, it is internalized, uncoated and reverse transcribed to form DNA. This permits insertion into the host genome and persistent gene expression, a major advantage of retroviral vectors. In addition, retroviral vectors can infect many different cell types. Some disadvantages include inactivation by complement, the requirement for dividing cells and a potential for insertional mutagenesis.

This mini-review was prepared from a presentation made at the World Congress of Gastroenterology, September 6 to 11, 1998, Vienna, Austria

¹Department of Medicine, Division of Gastroenterology-Hepatology, University of Connecticut School of Medicine, Farmington, Connecticut, USA;

²Shanghai Institute for Digestive Diseases, Shanghai Second Medical University, Renji Hospital, Shanghai, The People's Republic of China

Correspondence: Dr George Y Wu, Division of Gastroenterology-Hepatology, Herman Lopata Chair in Hepatitis, Research.

Telephone 860-679-3158, fax 860-679-3159, e-mail wu@nso.uhc.edu

Received for publication December 7, 1998. Accepted December 16, 1998

Recent advances in retroviral vectors: The long terminal repeat (LTR) regions of recombinant retroviral vectors contain viral enhancers and promoters that may activate host proto-oncogenes upon integration. Also, the enhancers can interfere with other promoters on the vector, making it difficult to manipulate the expression of the target gene. However, deletion of the LTR enhancer-promoter region reduces the virus titre greatly. Hwang et al (1) replaced the 3'-LTR enhancer-promoter region with a tetracycline operator. Viral expression was activated by providing a transactivator supplied from a plasmid in packaging cells, producing virus in high titre lacking the LTR.

In another approach, a self-deleting retroviral vector was prepared using a Cre-loxP system in which enhancers are self-deleted after integration (2).

Recent examples of in vivo gastrointestinal models for gene therapy: Shaked et al (3) transfected donor hepatocytes *ex vivo* by incubating them with retroviral vector encoding neomycin phosphotransferase or interleukin-7, resulting in sustained expression of target gene in hepatocytes following subsequent liver transplantation. In an *in vivo* transduction animal model, Kay et al (4) demonstrated that transfusion of recombinant retroviral vector encoding human factor IX into factor IX-deficient dogs via the portal vein resulted in persistent expression of factor IX for as long as five months. Noel et al (5) showed that retroviral vectors could transduce an intestine-derived cell line and that the efficiency was proportional to the amount of virus administered. Lau et al (6) investigated gene transfer in rat intestine by a retroviral vector encoding a reporter gene into the lumen of a segment of rat intestine and found that the reporter gene was expressed in both the crypts and villi.

Clinical trials: Grossman et al (7) studied a patient with familial hypercholesterolemia due to defective low density lipoprotein (LDL) receptors. A recombinant retroviral vector containing a normal gene for the LDL receptor was transduced into hepatocytes of the patient *ex vivo* and then transferred back into the body via portal vein infusion. The target gene was expressed for up to four months, with a significant decrease in LDL level and a decrease in the LDL to HDL ratio from 10 to 13 before treatment to 5 to 8 after treatment. These effects were maintained for at least 18 months.

Adenoviral Vectors

Strategy for use of adenoviruses as vectors: The main advantages of adenoviruses are that they do not require dividing cells and can infect a wide variety of tissues with high efficiency. In addition, the virus has a propensity for uptake by the liver. The genome generally remains in the nucleus as an extrachromosomal entity. However, expression of adenoviral vectors is usually transient. Early prototype adenoviral vectors caused inflammation and immune response upon repeated administration.

Recent advances in adenoviral vectors: Prototype adenoviral vectors caused an immune response against infected cells and could only transfer exogenous genes up to 8.5 kb in size. To address these problems, Haecker et al (8) developed a

new adenoviral delivery system with deletion of all viral genes. A helper virus was used to supply all essential viral genes for viral replication and packaging. The new vector successfully transferred target genes into myotubes *in vitro* and muscle fibres *in vivo*.

Recent examples of in vivo gastrointestinal models for gene therapy: Adenoviral vectors have been used to transfer target genes into primary human or murine hepatocytes via portal vein injection (9). A cDNA of human lipase was transferred by an adenovirus vector into gallbladder epithelial cell lines and also into isolated sheep gallbladder cells (10). In immunocompromised mice, intravenous injection of adenovirus-encoding reporter gene resulted in expression in colonic epithelium for six months (11). Also, there is evidence that adenoviral vectors can transfer a reporter gene into rat small intestine epithelial cells via an intestinal feeding tube (12).

Chimeric Adenoretroviral Vectors

A chimeric adenoretroviral vector combining the favourable aspects of adenoviruses and retroviruses was described by Bilbao et al (13). Two chimeric adenoretroviral vectors were prepared, by inserting sequences encoding retroviral packaging (LTR) and retroviral vector replication (retroviral proteins *gag/pol/env*) into separate adenoviral vectors. These two vectors were used to transfect target cells *in vivo*, converting the target cells into producer cells, which can generate progeny retroviral particles that infect neighbouring cells, with integration into the host genome and stable expression.

AAV Vectors

Strategy for use of AAVs as vectors: AAV vectors are prepared like adenoviruses in that the gene of interest is substituted for critical viral genes. However, preparation is more complicated, usually requiring co-infection of helper virus such as adenovirus or herpesvirus. Major advantages of this system are that the virus is nonpathogenic, does not require dividing cells and has a broad infection specificity, and that the wild-type AAV can specifically integrate into the host genome on chromosome 19. This has the potential of generating vectors with persistent expression through a predictable innocuous integration. However, recombinant AAV have been reported to integrate less specifically.

Recent examples of in vivo gastrointestinal models for gene therapy: Ponnazhagan et al (14) showed that intravenous injection of AAV resulted in a predominant uptake by the liver. Snyder et al (15) designed an AAV vector encoding the gene for human factor IX. The vector was prepared in a mutant adenovirus helper virus in which a missing early gene was supplied from a plasmid. A single infusion of AAV particles via portal vein into mice induced expression of human factor IX in the blood up to 2000 ng/mL for 36 weeks.

NONVIRAL VECTOR SYSTEMS

There are several nonviral vector delivery systems: direct injection of naked DNA, electroporation, particle bombard-

ment, calcium phosphate precipitation, liposome transfer and receptor-mediated transfer. Compared with viral methods, nonviral vectors are noninfectious, are generally less immunogenic, are less toxic to cells and can be modified to contain targeting components, but are also frequently less efficient.

LIPOSOMES

Strategy for use of liposomes as vectors: The most successful system consists of two elements – cationic lipid and negative-charged DNA fragment. The amphiphilic cationic lipid couples to negatively charged phosphate groups of DNA backbone, neutralizes the DNA charge and condenses the complex. Various kinds of lipids are applied in this system (16).

Recent examples of in vivo gastrointestinal models for gene therapy: Korba et al (17) used liposome-mediated phosphatidyl-ddG (2',3'-dideoxyguanosine, lamivudine) to inhibit the replication of hepatitis virus. Detected by HBV DNA, liposome-treated groups achieved a much lower than 90% effective concentration. Daily injection of such liposomes intraperitoneally into woodchuck hepatitis virus (WHV)-infected woodchucks for four weeks decreased serum WHV DNA by 23- to 46-fold compared with the group treated with free ddG.

Clinical trials: A phase I trial using a liposome system for in vivo hepatic delivery has been reported. Rubin et al (18) directly injected liposomes containing a human leukocyte antigen (HLA)-B7 gene with beta-2 microglobulin into hepatic metastatic lesions of 15 colorectal cancer patients. The target DNA was detected in 93% of patients, mRNA detected in 33%, and the HLA-B7 was detected in 50% to 63% (by fluorescence-activated cell sorting or immunohistochemistry, respectively). No serious toxicity was observed.

Ligand Receptor-mediated Gene Delivery Systems

In ligand receptor-mediated gene delivery systems, natural cell surface receptors are used to internalize nucleic acids by coupling an appropriate ligand with the DNA of interest. Depending on the specificity of the receptor for host cells, genes can be targeted to specific cell types. For example, asialoglycoproteins have been used to target genes to the liver by binding to the asialoglycoprotein receptor on the cell surface of hepatocytes. In an analogous manner, insulin and transferrin (19) have also been used to target genes to other cell types.

Asialoglycoproteins as vectors: The asialoglycoprotein receptor is selectively distributed on the plasma membrane of mammalian hepatocytes. After binding with the receptor, the asialoglycoprotein is internalized by endocytosis, entering an endosomal compartment, and ultimately lysosomes. By linking DNA noncovalently to an asialoglycoprotein carrier, DNA may be targeted to hepatocytes resulting in expression of the gene of interest.

Wu et al (20) showed that injection of an albumin-encoding plasmid complexed to an asialoglycoprotein increased albumin levels in albuminemic rats up to 37 µg/mL. Simi-

larly, an asialoglycoprotein/DNA complex was used to deliver the gene for a normal LDL receptor into Watanabe rabbit hepatocytes by peripheral venous injection. The cholesterol level in this animal model of familial hypercholesterolemia was decreased by 25% in serum, but the effect only lasted four to five days after which levels returned to previously high values (21).

To avoid intracellular degradation, approaches have been devised to incorporate agents that can disrupt endosomal vesicles, allowing DNA to escape before fusion with lysosomes. Viral fusogenic proteins such as influenza HA and vesicular stomatitis virus G protein (22,23) have been employed. Cristiano et al (24) co-infected primary cultures of murine hepatocytes with a replication-defective adenovirus together with an asialoglycoprotein-complexed reporter gene. All of the cells co-infected with adenovirus expressed the reporter gene, while only 0.1% of the cells without adenovirus were transfected. Similar results were also obtained in vivo. To ensure colocalization of the endosomolytic component and DNA complex, asialoglycoprotein was chemically linked to an adenovirus (25). This resulted in a modified adenovirus particle that had lost its natural infectivity but retained its infectivity for cells that possessed asialoglycoprotein receptors. Because of the known requirement of migration of endosomal vesicles on microtubules, a microtubule-disrupting drug, colchicine, was administered to block lysosomal degradation of targeted DNA. The gene for normal glucuronyl transferase in a targetable complex was injected intravenously following a single colchicine injection into Gunn rats with defective glucuronyl transferase. This resulted in substantial decreases in serum bilirubin and production of bilirubin conjugates in bile that lasted for at least 60 days (26).

MOLECULAR STRATEGIES FOR INHIBITION OF PRE-EXISTING GENE EXPRESSION

Chimeric Molecules

An RNA/DNA hybrid capable of correcting a genetic mutation in situ has been described. These hybrids contained RNA nucleotides that were modified by 2'-O-methylation on the ribose to prevent degradation by nuclease. An RNA/DNA chimeric was successfully introduced into the Huh-7 hepatoma cell line to correct a mutation in an alkaline phosphatase gene with reported efficiency of 30% (27). More recently, using a chimeric oligonucleotide, a mutation was produced in a factor IX gene in cell culture and in vivo. The latter resulted in a prolongation of the activated partial thromboplastin time (28).

Ribozymes

Ribozymes are catalytic RNA molecules that can specifically bind and cleave a target RNA (29). Chemical modification can enhance the stability of ribozymes. Hammerhead ribozymes have been prepared against the hepatitis C virus (HCV) targeting its 5'-noncoding and core domains (30). In vitro assays showed that those ribozymes specifically cleaved target sequences of HCV and suppressed the expression of a

reporter gene by up to 71%. Ribozymes have been introduced into hepatitis B virus (HBV)-, HCV- infected cells (31,32) to block the transcription of viral proteins.

The 5', 3' region around the core catalytic domain of ribozymes may affect its activity. Ruiz et al (33) created a multimeric ribozyme molecule to solve this problem. A monomer of ribozyme was designed in which target regions to the hepatitis B virus were placed 5' and 3' to the catalytic domain of the ribozyme. The construct was then cloned as tandem into an expression vector. Upon transfection, self-cleavage occurred, producing multiple monomeric ribozymes, lacking any extra sequences around its catalytic region.

Because ribozymes are composed of RNA, they are quite susceptible to nuclease degradation. This property can limit the effectiveness in cells. A DNA equivalent of ribozymes that will cleave at the direct repeat I and polyadenylation signal regions of the HBV mRNA was prepared. This enzyme was shown to inhibit intracellular HBV gene expression substantially, and can be modified to increase stability while maintaining activity (34).

Antisense Oligonucleotides

Antisense oligonucleotides are synthetic molecules designed to hybridize with a target mRNA sequence and block its translation. Antisense oligonucleotides in the form of asialoglycoprotein targetable complexes when introduced into HBV-producing cells significantly suppressed the viral gene expression and replication in vitro. Furthermore, pretreatment of Huh-7 hepatoma cells with asialoglycoprotein-based complexes of an antisense against the HBV poly A signal and 5' upstream region, blocked the subsequent HBV production after transfection of a plasmid that contained the complete HBV genome. Viral RNA was decreased by 60%, and the expression of hepatitis B surface antigen decreased by 97% (35).

Antisense oligodeoxynucleotides against duck HBV (DHBV) were prepared by Offensperger et al (36). Primary hepatocytes from DHBV-infected ducklings were exposed to these antisense oligonucleotides, resulting in strong inhibition of DHBV replication in vitro. Assays on two-week DHBV postinfected ducks showed that intravenous infusion of antisense oligodeoxynucleotides at a daily dose of 20 µg/g body weight for 10 consecutive days almost totally abolished DHBV replication. Also, injection of such antisense oligonucleotides into newborn healthy ducklings prevented subsequent infection by DHBV when challenged with infectious virus.

Using another model, Bartholomew et al (37) showed that targeted delivery of an antisense sequence against the poly A signal and 5' upstream region of WHV using a complex with an asialoglycoprotein-based conjugate in a woodchuck hepatitis model significantly decreased the virus particles in the bloodstream by five- to 10-fold, and this decrease was maintained over two weeks.

The 5' nontranslated region (NTR) of HCV contains important elements that control HCV translation. Wu and Wu (38) prepared antisense oligonucleotides directed against a

sequence in the internal ribosomal binding site of the NTR, and a portion of the NTR overlapping the core protein translational start site of HCV. In transient transfections of a plasmid containing a luciferase gene immediately downstream from an HCV NTR insert, oligonucleotides in the form of asialoglycoprotein-polylysine complexes were administered to Huh-7 cells, and luciferase activity generated by cytomegalovirus HCVluc measured. Antisense complexes inhibited luciferase activity by 75% and 99% at 0.01 µM and 0.1 µM, respectively. In cell lines stably transfected with cytomegalovirus HCVluc plasmid, complexed antisense complexes inhibited luciferase activity in Huh-7 cells by 20% at 10 µM and 85% at 60 µM, and were competeable by an excess asialoglycoprotein (38).

Dominant Negative Gene Products

A dominant negative gene product is a mutant protein that interacts and interferes with the function of its normal counterpart. This phenomenon was discovered as a natural mutant of hepadnaviral core protein, which inhibited the function of wild-type core protein and inhibited replication of the virus. Subsequently, intentional core mutants of human HBV and WHV were shown to block wild-type viral replication by 95%. By using retroviral or adenoviral vectors, Scaglioni et al (39) transferred HBV- or DHBV-dominant negative core mutants into hepatoma cell lines that had either constitutive or transient hepatitis infection, viral replication was greatly inhibited.

REFERENCES

- Hwang JJ, Li L, Anderson WF. A conditional self-inactivating retrovirus vector that uses a tetracycline-responsive expression system. *J Virol* 1997;71:7128-31.
- Russ AP, Friedel C, Grea M, von Melchner H. Self-deleting retrovirus vectors for gene therapy. *J Virol* 1996;70:4927-32.
- Shaked A, Csete ME, Shiraiishi M, et al. Retroviral-mediated gene transfer into rat experimental liver transplant. *Transplantation* 1994;57:32-4.
- Kay MA, Rothenberg S, Landen CN, et al. In vivo gene therapy of hemophilia B: sustained partial correction in factor IX-deficient dogs. *Science* 1993;262:117-9.
- Noel RA, Shukla P, Henning SJ. Optimization of gene transfer into intestinal epithelial cells using a retroviral vector. *J Pediatr Gastroenterol Nutr* 1994;19:43-9.
- Lau C, Soriano HE, Ledley FD, et al. Retroviral gene transfer into the intestinal epithelium. *Hum Gene Ther* 1995;6:1145-51.
- Grossman M, Raper SE, Kozarsky K, et al. Successful ex vivo gene therapy directed to liver in a patient with familial hypercholesterolaemia. *Nat Genet* 1994;6:335-41.
- Haecker SE, Stedman HH, Balice-Gordon RJ, et al. In vivo expression of full-length human dystrophin from adenoviral vectors deleted of all viral genes. *Hum Gene Ther* 1996;7:1907-14.
- Morsy MA, Alford EL, Bett A, Graham FL, Caskey CT. Efficient adenoviral-mediated ornithine transcarbamylase expression in deficient mouse and human hepatocytes. *J Clin Invest* 1993;92:1580-6.
- Maeda H, Danel C, Crystal RG. Adenovirus-mediated transfer of human lipase complementary DNA to the gallbladder. *Gastroenterology* 1994;106:1638-44.
- Brown GR, Thiele DL, Silva M, Beutler B. Adenoviral vectors given intravenously to immunocompromised mice yield stable transduction of the colonic epithelium. *Gastroenterology* 1997;112:1586-94.
- Cheng DY, Kolls JK, Lei D, Noel RA. In vivo and in vitro gene transfer and expression in rat intestinal epithelial cells by E1-deleted adenoviral vector. *Hum Gene Ther* 1997;8:755-64.
- Bilbao G, Feng M, Rancourt C, Jackson WH, Curiel DT.

- Adenoviral/retroviral vector chimeras: a novel strategy to achieve high-efficiency stable transduction in vivo. *FASEB J* 1997;11:624-34.
14. Ponnazhagan S, Mukherjee P, Yoder MC, et al. Adeno-associated virus2-mediated gene transfer in vivo: organ-tropism and expression of transduced sequences in mice. *Gene* 1997;190:203-10.
 15. Synder RO, Miao CH, Patijn GA, et al. Persistent and therapeutic concentrations of human factor IX in mice after hepatic gene transfer of recombinant AAV vectors. *Nat Genet* 1997;16:270-6.
 16. Mahato RI, Rolland A, Tomlinson E. Cationic lipid-based gene delivery systems: pharmaceutical perspectives. *Pharm Res* 1997;14:853-9.
 17. Korba BA, Xie H, Wright KN, et al. Liver-targeted antiviral nucleotides: Enhanced antiviral activity of phosphatidyl-dideoxyguanosine versus dideoxyguanosine in woodchuck hepatitis virus infection in vivo. *Hepatology* 1996;23:958-63.
 18. Rubin J, Galanis E, Pitot HC, et al. Phase I study of immunotherapy of hepatic metastases of colorectal carcinoma by direct gene transfer of an allogeneic histocompatibility antigen, HLA-B7. *Gene Ther* 1997;4:19-25.
 19. Cotten M, Langle-Rouault F, Kirlappos H, et al. Transferrin-polycation-mediated introduction of DNA into human leukemic cells: stimulation by agents that affect the survival of transfected DNA or modulate transferrin receptor levels. *Proc Natl Acad Sci USA* 1990;87:4033-7.
 20. Wu GY, Wilson JM, Shalaby F, Grossman M, Shafritz DA, Wu CH. Receptor-mediated gene delivery in vivo. Partial correction of genetic ananalbuminemia in Nagase rates. *J Biol Chem* 1991;266:14338-42.
 21. Wilson JM, Grossman M, Wu CH, Chowdhury NR, Wu GY, Chowdhury JR. Hepatocyte-directed gene transfer in vivo leads to transient improvement of hypercholesterolemia in low density lipoprotein receptor-deficient rabbits. *J Biol Chem* 1992;267:963-7.
 22. Schoen P, Corver J, Meijer DK, Wilschut J, Swart PJ. Inhibition of influenza virus fusion by polyanionic proteins. *Biochem Pharmacol* 1997;53:995-1003
 23. Lowy RJ, Covell D, Eidelman O, Walter A, Blumenthal R. Activation of vesicular stomatitis virus fusion with cells by pretreatment at low pH. *J Biol Chem* 1988;263:4749-53.
 24. Cristiano RJ, Smith LC, Woo SLC. Hepatic gene therapy: adenovirus enhancement of receptor-mediated gene delivery and expression in primary hepatocytes. *Proc Natl Acad Sci USA* 1993;90:2122-6.
 25. Wu GY, Zhan P, Sze LL, Rosenberg AR, Wu CH. Incorporation of adenovirus into a ligand-based DNA carrier system results in retention of original receptor specificity and enhances targeted gene expression. *J Biol Chem* 1994;269:11542-6.
 26. Chowdhury NR, Hays RM, Bommineni VR, et al. Microtubular disruption prolongs the expression of human bilirubinuridinephosphogluconate-glucuronosyltransferase-1 gene transferred into Gunn rat livers. *J Biol Chem* 1996;271:2341-6.
 27. Kren BT, Cole-Strauss A, Kmiec EB, Steer CJ. Targeted nucleotide exchange in the alkaline phosphatase gene of HuH-7 cells mediated by a chimeric RNA/DNA oligonucleotide. *Hepatology* 1997;25:1462-8.
 28. Kren BT, Bandyopadhyay P, Steer CJ. In vivo site-directed mutagenesis of the factor IX gene by chimeric RNA/DNA oligonucleotides. *Nat Med* 1998;4:285-90.
 29. Cech TR, Zaug AJ, Grabowski PJ. In vitro splicing of the ribosomal RNA precursor of Tetrahymena: Involvement of a guanosine nucleotide in the excision of the intervening sequence. *Cell* 1981;29:487-96.
 30. Sakamoto N, Wu CH, Wu GY. Intracellular cleavage of hepatitis C virus RNA and inhibition of viral protein translation by hammerhead ribozymes. *J Clin Invest* 1996;98:2720-8.
 31. von Weizsacker F, Blum HE, Wands JR. Cleavage of hepatitis B virus RNA by three ribozymes transcribed from a single DNA templates. *Biochem Biophys Res Commun* 1992;189:743-8.
 32. Beck J, Nassal M. Efficient hammerhead ribozyme-mediated cleavage of the structured hepatitis B virus encapsidation signal in vitro and in cell extract, but not in intact cells. *Nucleic Acids Res* 1995;23:4954-62
 33. Ruiz J, Wu CH, Ito Y, Wu GY. Design and preparation of a multimeric self-cleaving hammerhead ribozyme. *Biotechniques* 1997;22:338-45.
 34. Asahina Y, Ito Y, Wu CH, Wu GY. DNA ribonucleases that are active against intracellular hepatitis B viral RNA targets. *Hepatology* 1998;28:547-54.
 35. Nakazono K, Ito Y, Wu CH, Wu GY. Inhibition of hepatitis B virus replication by targeted pretreatment of complexed antisense DNA in vitro. *Hepatology* 1996;23:1297-303.
 36. Offensperger WB, Offensperger S, Walter E, et al. In vivo inhibition of duck hepatitis B virus replication and gene expression by phosphorothioate modified antisense oligodeoxynucleotides. *EMBO J* 1993;12:1257-62.
 37. Bartholomew RM, Carmichael EP, Findeis MA, Wu CH, Wu GY. Targeted delivery of antisense DNA in woodchuck hepatitis virus-infected woodchucks. *J Viral Hepat* 1995;2:273-8.
 38. Wu CH, Wu GY. Targeted inhibition of hepatitis C virus-directed gene expression in human hepatoma cell lines. *Gastroenterology* 1998;114:1304-12.
 39. Scaglioni P, Melegari M, Takahashi M, Chowdhury JR, Wands J. Use of dominant negative mutants of the hepadnaviral core protein as antiviral agents. *Hepatology* 1996;24:1010-7.



Hindawi
Submit your manuscripts at
<http://www.hindawi.com>

