GENE THERAPY FOR LIVER DISEASES

Experimental Strategies
GENE THERAPY

- Principles
- Experimental/ preclinical applications
- Perspectives
LIVER DISEASES
Genetic Classification

- Hereditary monogenic
- Acquired monogenic
- Polygenic
LIVER DISEASES
Genetic Classification

• Hereditary monogenic
  – Crigler-Najjar syndrome type 1
  – Alpha-1-antitrypsin deficiency
  – Wilson disease
  – Hereditary hypercholesterolemia
  – Hemophilia A and B

• Acquired monogenic
  – Viral hepatitis A-E
  – HCC

• Polygenic
  – Liver cirrhosis
  – HCC
LIVER DISEASES
Gene Therapy Strategies

• Hereditary monogenic
  – Gene repair
  – Gene replacement
  – Cell or liver transplantation

• Acquired monogenic
  – Block of gene expression
  – DNA vaccination

• Polygenic
  – Gene augmentation
  – DNA vaccination
Correction of the UDP-glucuronosyltransferase gene defect in the Gunn rat model of Crigler–Najjar syndrome type I with a chimeric oligonucleotide

Betsy T. Kren*, Bhupesh Parashar†, Paramita Bandyopadhyay*, Namita Roy Chowdhury†‡, Jayanta Roy Chowdhury†‡, and Clifford J. Steer*§¶
CRIGLER-NAJJAR SYNDROME TYPE I
Gene Defect

C deletion

5'AGCTGGGATTGACCCTGAATGTCTTGAAAATGACTGCAGCGATGATTGGTCGACCCACTGGGACTTACAGGACCTTTACTGACGGCTACTAAAC
3'TCGACCCACTGGGACTTACAGGACCTTTACTGACGGCTACTAAAC

....Glu-Met....

C deletion

....Lys-STOP...
CRIGLER-NAJJAR SYNDROME TYPE I
Gene Repair - Chimeric Oligonucleotide

5’AGCTGGGGTGACCTGAAATGTGACGCGATGATTGG3’
TGCCTGG-gggacuuacaggAcCCTTAacugacgccuatT
5’3’5’
TGCCTGCTCCTGAAATGTGACGCGATT
3’TCGAAnnCnACTGGGACTTACAGGACTTACTGACGGCTACTAAnC5’
5’AGCTGGGGTGACCTGAAATGTGACGCGATGATTGG
TCGAAnnCnACTGGGACTTACAGGACTTACTGACGGCTACTAAnC

.....Lys-STOP

.....Glu-Met.....

.....Glu-Met.....
CRIGLER-NAJJAR SYNDROME TYPE I
Gene Repair - Chimeric Oligonucleotide

Kren BT et al. Proc Natl Acad Sci USA 1999;96:10349-10354
HBV - HCV INFECTION
The diagram illustrates the process of gene expression. It consists of five steps:

1. **DNA** (Deoxyribonucleic Acid) contains the genetic information.
2. **Transcription** involves the synthesis of messenger RNA (mRNA) from the DNA template.
3. **mRNA** then undergoes translation into a sequence of amino acids.
4. **Translation** results in the formation of a protein.

The genetic code is represented by nucleotides (A, T, G, C) and amino acids (Lys, Pro, Leu, Cys, Asp). The mRNA sequence is shown as a string of nucleotides, indicating the reading frame for protein synthesis.
Small RNAs Make Big Splash

Just when scientists thought they had deciphered the roles played by the cell’s leading actors, a familiar performer has turned up in a stunning variety of guises. RNA, long upstaged by its more glamorous sibling, DNA, is turning out to have star qualities of its own.

NOBEL PRIZE 2006
FOR PHYSIOLOGY AND MEDICINE

Interfering RNA

Andrew Fire and Craig Mellow
SMALL INTERFERING RNA (siRNA)

(RNA Induced Silencing Complex)

1. siRNA
2. ATP → ADP
3. Helicase
4. Nuclease

Degradation by cellular RNases

Cellular nucleases

Target mRNA

Inhibition of hepatitis B virus in mice by RNA interference

Anton P McCaffrey¹, Hiroyuki Nakai¹,⁴, Kusum Pandey¹,⁴, Zan Huang¹,⁴, Felix H Salazar², Hui Xu¹, Stefan F Wieland³, Patricia L Marion² & Mark A Kay¹
HBV INFECTION
siRNA - Mouse HBsAg

HBV INFECTION
siRNA - Mouse HBcAg

CONTROL   CONTROL   siRNA

Clearance of replicating hepatitis C virus replicon RNAs in cell culture by small interfering RNAs

Glenn Randall, Arash Grakoui, and Charles M. Rice*

Proc Natl Acad Sci USA 2003;100;235-240
HCV REPLICON INHIBITION
NS5B siRNA

siRNA: siIRR     siHCV
      siHCV Sense A/S

NS5B

B-Actin

Randall G et al. Proc Natl Acad Sci USA 2003;100;235-240
Genetic Immunization of Chimpanzees Chronically Infected with the Hepatitis B Virus, Using a Recombinant Retroviral Vector Encoding the Hepatitis B Virus Core Antigen

MATTI SÄLLBERG,¹,² JANICE HUGHES,³ ALI JAVADIAN,⁴ GREG RONLOV,¹ CATHARINA HULTGREN,² KAY TOWNSEND,¹ CAROL-GAY ANDERSON,¹ JOANNE O’DEA,¹ JEAN ALFONSO,¹ ROBERT EASON,⁴ KRISHNA K. MURTHY,⁵ DOUGLAS J. JOLLY,¹ STEPHEN M. CHANG,¹,⁶ STEVEN J. MENTO,¹,⁷ DAVID MILICH,³ and WILLIAM T. LEE¹

Human Gene Ther 1998;9:1719-1729
HBV THERAPY
DNA Vaccination

Hepatocyte growth factor gene therapy of liver cirrhosis in rats

Takahiro Ueki¹, Yasufumi Kaneda², Hiroko Tsutsui³, Kenji Nakanishi³, Yoshiki Sawa⁴, Ryuichi Morishita⁵, Kunio Matsumoto⁶, Toshikazu Nakamura⁶, Hiroshi Takahashi⁷, Eizo Okamoto¹ & Jiro Fujimoto¹

Nat Medicine 1999;5:226-230
HGF GENE THERAPY
DMN Liver Cirrhosis

DMN: ▲▲▲▲▲▲▲▲▲
HGF-HVJ liposome or PBS: ▲ ▲ ▲ ▲

HGF GENE THERAPY
Cirrhotic Rats - Survival

HGF GENE THERAPY
Reversal of Liver Cirrhosis

DMN (wk 6)
DMN + HGF (wk 6)
DMN + HGF (wk 8)

Tumor Suppressor Genes

Novel 'Targeted' Drugs

Suicide Genes

Oncolytic Viruses

Immune Therapy - DNA Vaccination
- Tumor specific genes, e.g., AFP
- Cytokine genes, e.g., IL-2, IL-12
- Dendritic cells
- Tumor infiltrating lymphocytes

Anti-Angiogenic Genes
Complete Regression of Established Murine Hepatocellular Carcinoma by In Vivo Tumor Necrosis Factor α Gene Transfer

GUANGWEN CAO,* SHIGEKI KURIYAMA,† PING DU,* TAKEMI SAKAMOTO,† XIANTAO KONG,* KAZUHIRO MASUI,† and ZHONGTIAN QI*

*Department of Microbiology, Second Military Medical University, Shanghai, China; and †Third Department of Internal Medicine, Nara Medical University, Kashihara, Nara, Japan

Gastroenterology 1997;112:501-510
HCC GENE THERAPY

TNF-α

Tumor Diameter (mm)

Day

Vector
SV40-TNF-α
Alb-TNF-α

Cao G et al. Gastroenterology 1997;112:501-510
HCC GENE THERAPY

TNF-α

Vector

SV40-TNF-α

Alb-TNF-α

Cao G et al. Gastroenterology 1997;112:501-510
Antiangiogenic Gene Therapy for Hepatocellular Carcinoma Using Angiostatin Gene

Hiroki Ishikawa,¹ Kazuhiko Nakao,² Kojiro Matsumoto,¹ Tatsuki Ichikawa,¹ Keisuke Hamasaki,¹ Keisuke Nakata,¹ and Katsumi Eguchi¹

Hepatology 2003;37:696-704
HCC GROWTH
Non-Transfected/ Transfected Cells
PREVENTION OF LIVER DISEASES & HCC

1. Acute Hepatitis
2. Chronic Hepatitis
3. Cirrhosis
4. HCC in Cirrhosis
A T-cell HCV vaccine eliciting effective immunity against heterologous virus challenge in chimpanzees

Antonella Folgori, Stefania Capone, Lionello Ruggeri, Annalisa Meola, Elisabetta Sporeno, Bruno Bruni Ercole, Monica Pezzanera, Rosalba Tafi, Mirko Arcuri, Elena Fattori, Armin Lahm, Alessandra Luzzago, Alessandra Vitelli, Stefano Colloca, Riccardo Cortese & Alfredo Nicosia
HEPATITIS C
T Cell Vaccine

HEPATITIS C T Cell Vaccine

Vaccine Group

Control Group

HCV RNA
ALT
GGT

HCV RNA
ALT
GGT

HCV RNA
ALT
GGT

HCV RNA
ALT
GGT

Folgori A et al Nat Med 2006; 12:190-197
LIVER DISEASES
Gene Therapy - Summary

• Gene repair
  Crigler-Najjar syndrome type 1

• Block of gene expression and DNA vaccination
  Hepatitis B and C

• Gene augmentation
  Liver cirrhosis and HCC

• Molecular prevention
  HBV and HCV infection