[S2-3] [10/17/2002 (Thurs) 11:50~12:20/Hall B]

Cell signaling and therapeutic drug target for the treatment of liver cirrhosis

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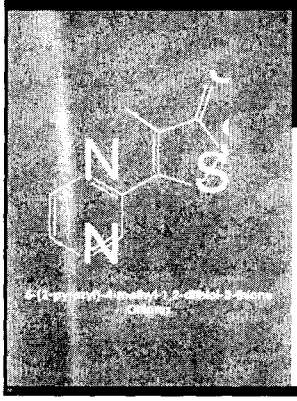
Signal transduction refers to the processess by which cells perceive the environment and/or internal status. In many physiological responses, cellular signals are activated by the transducers attached to the cell surface plasma membrane in response to chemical modulators. Advances in information technology are required in the pharmaceutical sciences to screen and explore the potential therapeutic agents. Liver cirrhosis is a chronic disease with high mortality rate. In the United States and Western world as well as Oriental countries, liver cirrhosis is the major leading cause of death by disease. Yet, no effective therapeutic agent is available for liver cirrhosis treatment.

Hepatocyte growth factor (HGF), a ligand of c-Met receptor, stimulates activation of cellular kinases via phosphatidylinositol 3-kinase (PI3-kinase). It has been proposed that repeated transduction of skeletal muscles with hepatocyte growth factor gene is potentially useful for the treatment of liver cirrhosis in an animal model. CCAAT/enhancer binding protein (C/EBP) controls cell cycle progression. We determined whether HGF activates C/EBP in association with the S phase entrance of cell replication and whether PI3-kinase contributes to the activation of C/EBP. Treatment of H4IIE cells, a hepatocyte-derived cell line, with HGF increased C/EBP DNA binding activity at an early time. Immunodepletion, subcellular fractionation and confocal microscopic analyses revealed that the HGF-induced C/EBP DNA binding activity depended on nuclear translocation of C/EBPβ. Whereas stable transfection of p110 catalytic subunit of PI3-kinase enhanced HGF-mediated nuclear translocation of C/EBPβ and DNA binding, stable transfection of p85 subunit or a chemical inhibitor of PI3-kinase completely blocked C/EBP activation. HGF increased luciferase reporter activity in cells transfected with a mammalian cell expression vector containing –1.65 kb rGSTA2 promoter comprising C/EBP response element (pGL-1651). Whereas transfection with pCMV500 a control vector allowed pGL-1651 to respond to HGF, expression of dominant-negative mutant C/EBP completely inhibited the ability of HGF to stimulate the reporter gene expression. Stable transfection experiments with p110 and p85 subunits of PI3-kinase revealed that the increase in cell growth rate by HGF was dependent on PI3-kinase. Hence, HGF induces nuclear translocation of C/EBPβ via the PI3-kinase pathway, and stimulates C/EBP DNA binding and gene transcription, and that the PI3-kinase-mediated C/EBP

activation may contribute to cell replication. In spite of the potential use of HGF for liver cirrhosis, the approach of HGF gene therapy is limited due to problems in targeted DNA delivery, the duration of transgene expression and adverse consequence of heterologous gene expression.

Oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione] has been used clinically and is of little toxicity. Comprehensive mechanistic and phase IIa clinical studies supported the notion that oltipraz exerts chemopreventive effects against chemical carcinogenesis. Laboratory cirrhotic rats produced by dimethylnitrosamine administrations simulate the clinical features of human liver cirrhosis such as mortality, ascites, hepatic parenchymal cell destruction, formation of connective tissue and nodular regeneration, providing a preclinical model to evaluate therapeutic efficacy of drug and underlying mechanism. Oltipraz was orally administered to cirrhotic rats for 4 weeks and the survival rate was monitored. Surviving animals were subjected to blood biochemical, liver histopathological and immunochemical analyses. Primary cultured hepatic stellate cells or hepatocytes were used for detransactivation experiment or immunocytochemical assay. Treatment of cirrhotic rats with oltipraz significantly increased the survival rate and body weight gain. The low plasma albumin level in cirrhotic rats was restored by oltipraz treatment along with a reduction of ascites. Oltipraz decreased both the accumulation of extracellular matrix and the formation of multiple liver fibrotic nodules. Consistent with this, oltipraz treatment eliminated α-smooth muscle actin (α-SMA)-positive cells, intensely visualized in the vicinity of fibers in cirrhotic liver. In activated stellate cells, oltipraz inhibited α-SMA expression. Oltipraz activated CCAAT/enhancer binding protein (C/EBP)β in stellate cells, which was responsible for the suppression of transforming growth factor-β1 expression. The number of proliferating hepatocytes was increased by oltipraz in the liver of cirrhotic rats, while that of Thy1.1- or Flt-3-positive undifferentiated cells was decreased. In cultured hepatocytes, oltipraz activated C/EBPs, key transcription factors for liver regeneration. Compatibly, oltipraz restored the expression of down-regulated C/EBP in cirrhotic rats with an increase in liver weight. We report that oltipraz within the clinical dose range regenerates cirrhotic liver in rats with the established liver cirrhosis as a result of reduction of the intensities of cirrhotic nodules, elimination of accumulated extracellular matrix and inactivation of stellate cells, thereby improving the survival rate. We also reveal that activation of CCAAT/enhancer binding protein by oltipraz inhibits transforming growth factor β1 gene expression in stellate cells.

Understanding of the interplay among parenchymal and nonparenchymal cells in the cirrhotic liver will provide cellular and molecular target(s) for pharmacological treatment of liver cirrhosis. We identify the best targets and devise the approaches to the modulation of cell function for therapeutic advantages.



Cell Signaling and Therapeutic Drug Targets for Treatment of Liver Cirrhosis

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Liver Cirrhosis
A Fatal Liver Disease

- **The unhealthy rate of male salary men is double of that in female in Korea (23%): Liver problems are the major contributing factor. The proportion of men with potential liver dysfunction is 13% (One out of 6 men).**
  Korean Health Insurance, 2000. 1

- **The morbidity rate of liver cirrhosis among the male patients in the fifties with liver diseases is 20%.**
  Ministry of Health and Welfare, 1999. 12

- **No efficacious therapeutic agent is available yet.**

	Korea	Worldwide	
# of Patients (2000, WHO)	0. 10 million	12 million	Mortality rate in USA: 25,000 per year (Vital & Health Statistics 13, No. 145)

Stages of Liver Cirrhosis

Healthy Liver

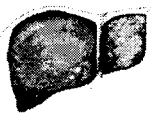
-Absorption of nutrients
-Storage of energy source
-Detoxification
-A silent organ

Chronic Hepatitis

-Proliferation of HBV
-Activation of immune system
-Hepatocyte death
-Reversible fibrosis

Liver Cirrhosis

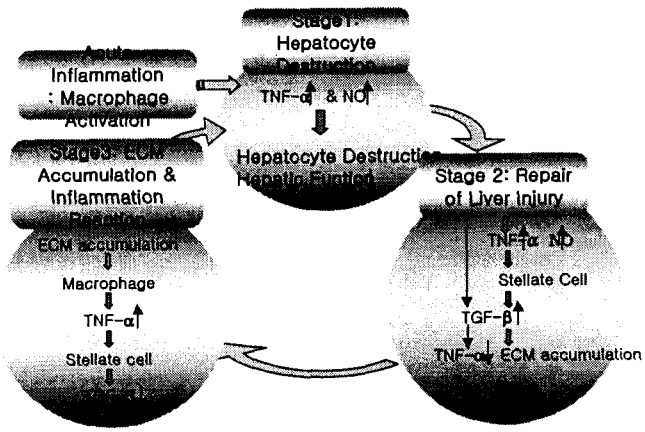
-Hepatic malfunction
-Transplantation ?

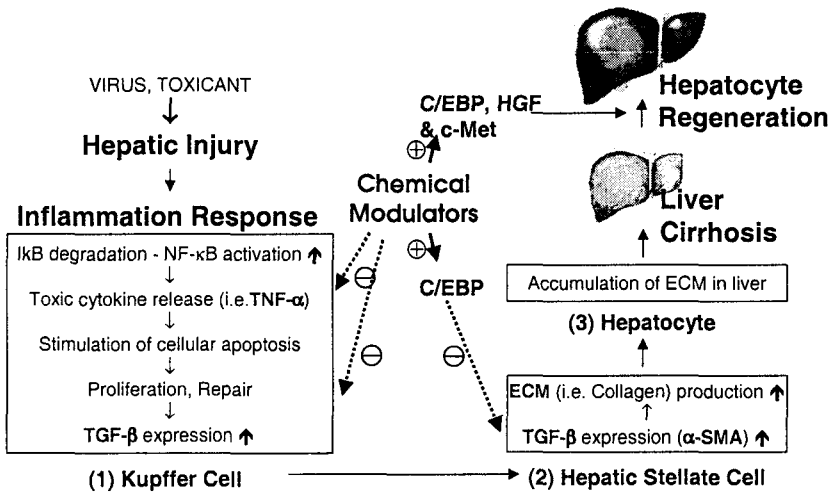


Virus, Parasite,
Heavy drinking,
Chemicals

Death

Malignant Loop of Hepatic Fibrogenesis





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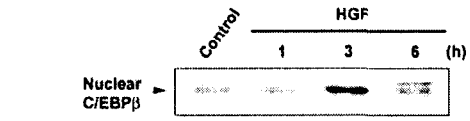
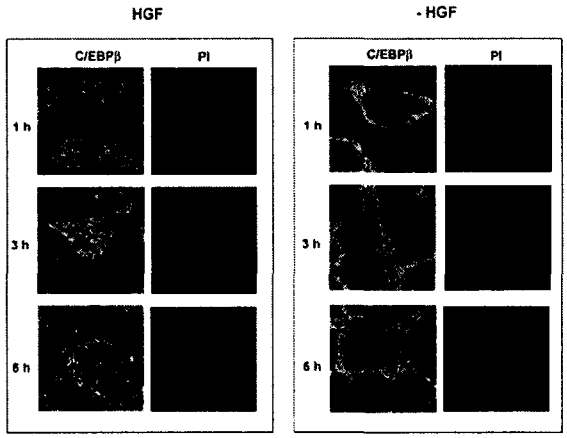
Hepatocyte growth factor gene therapy of liver cirrhosis in rats

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

C/EBPβ-mediated liver regeneration by HGF

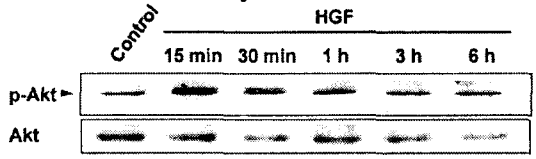
Stimulation of C/EBPβ translocation into the nucleus by HGF



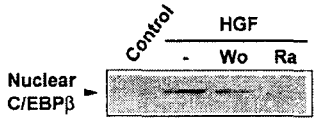


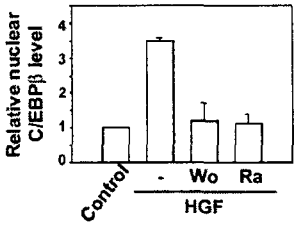
PI3-kinase-dependent C/EBP translocation by HGF

Activation of PI3-kinase by HGF

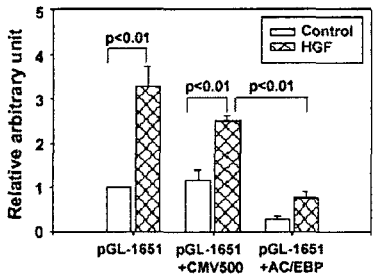


The role of PI3 kinase on C/EBP translocation by HGF

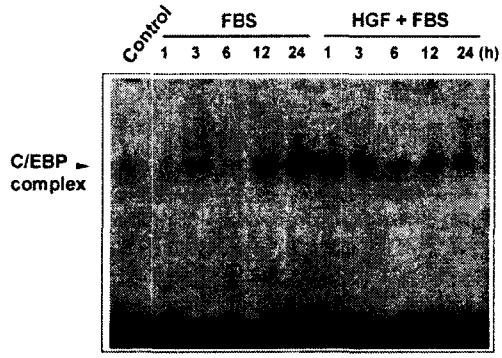


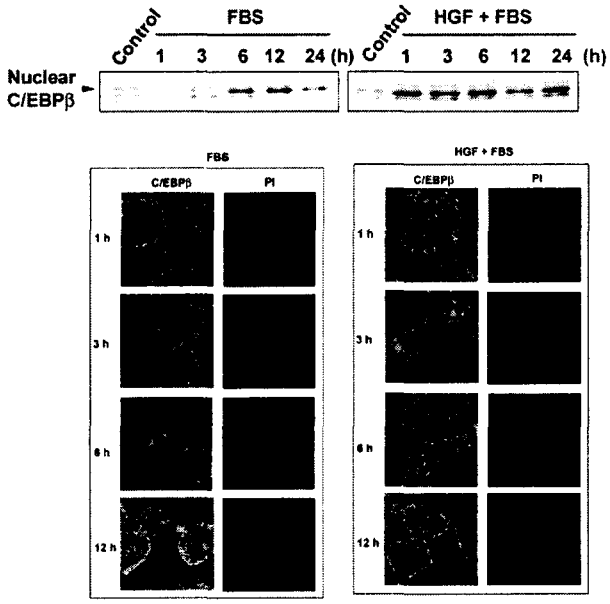


Inhibition of HGF-inducible reporter gene activation by dnC/EBP.

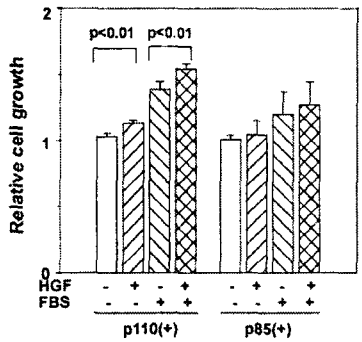


Effect of HGF + FBS on the activation of C/EBP



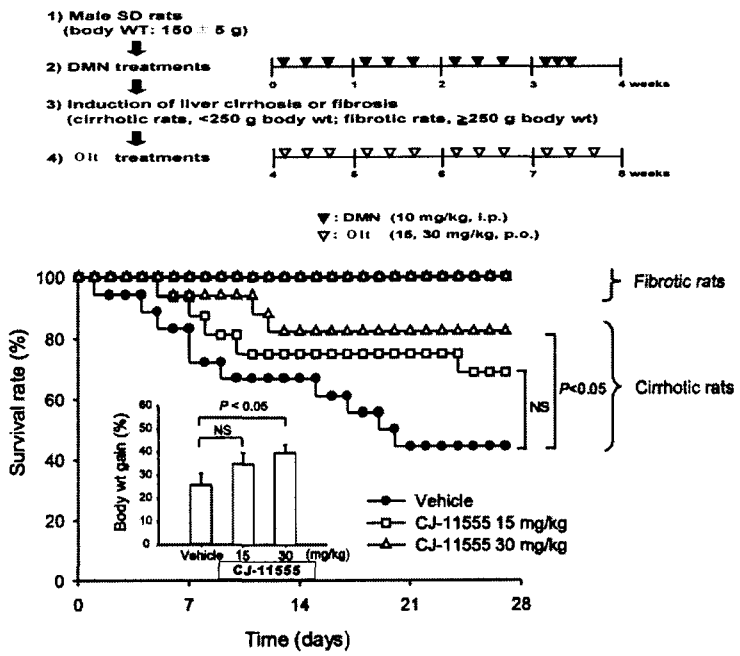


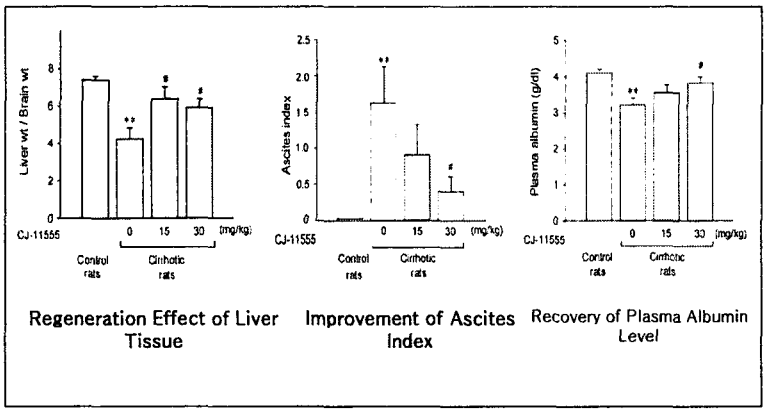
PI3-kinase-dependent cell growth by HGF



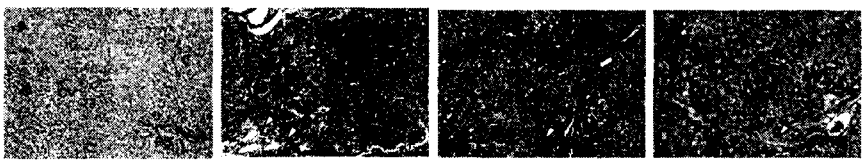
PI3-Kinase-mediated C/EBPβ activation plays a role in the liver growth and differentiation by HGF

C/EBPβ-mediated liver regeneration by CJ11555 (Oltipraz)





Regeneration Effect of Liver Tissue | Improvement of Ascites Index | Recovery of Plasma Albumin Level

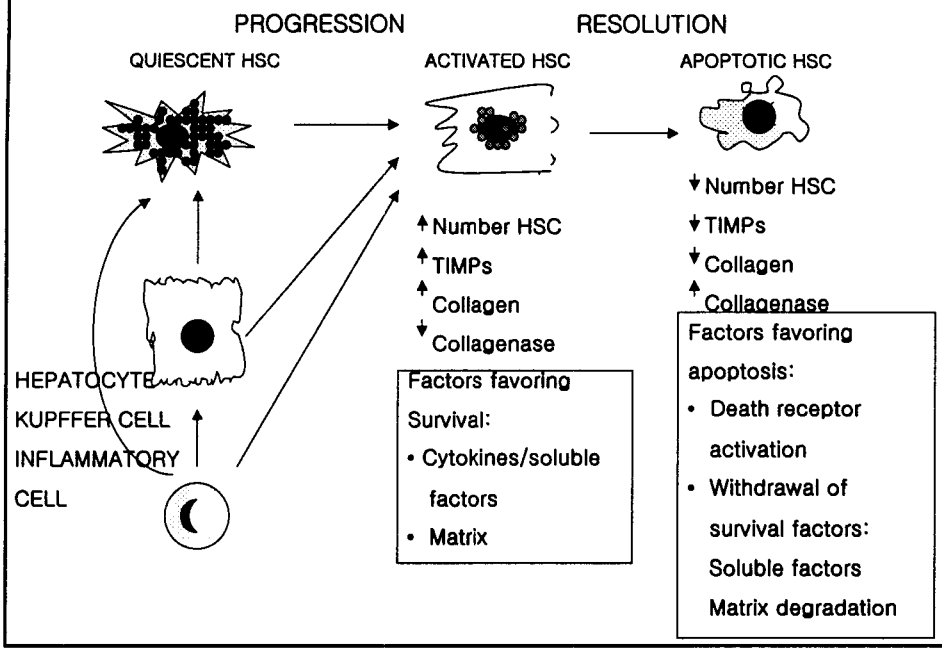


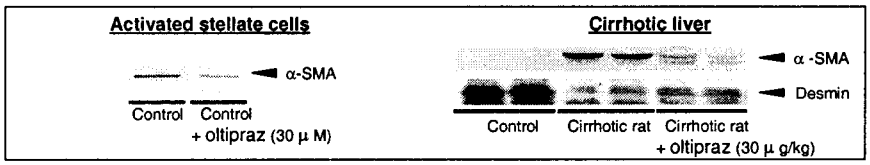
Control | Cirrhotic rat | + oltipraz (15 mg/kg) | + oltipraz (30 mg/kg)

Group	Control	Cirrhotic rat	+ oltipraz (15 mg/kg)	+ oltipraz (30 mg/kg)
Fibrosis Score	0	3.8 ± 0.2	2.9 ± 0.4	2.8 ± 0.3*
Knodell Score	0	14.0 ± 0.8	8.7 ± 1.1**	6.8 ± 2.5**

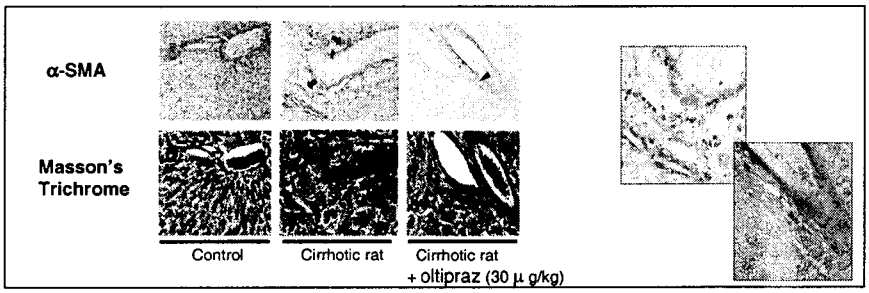
(*p<0.05, **p<0.01)

FIBROGENESIS

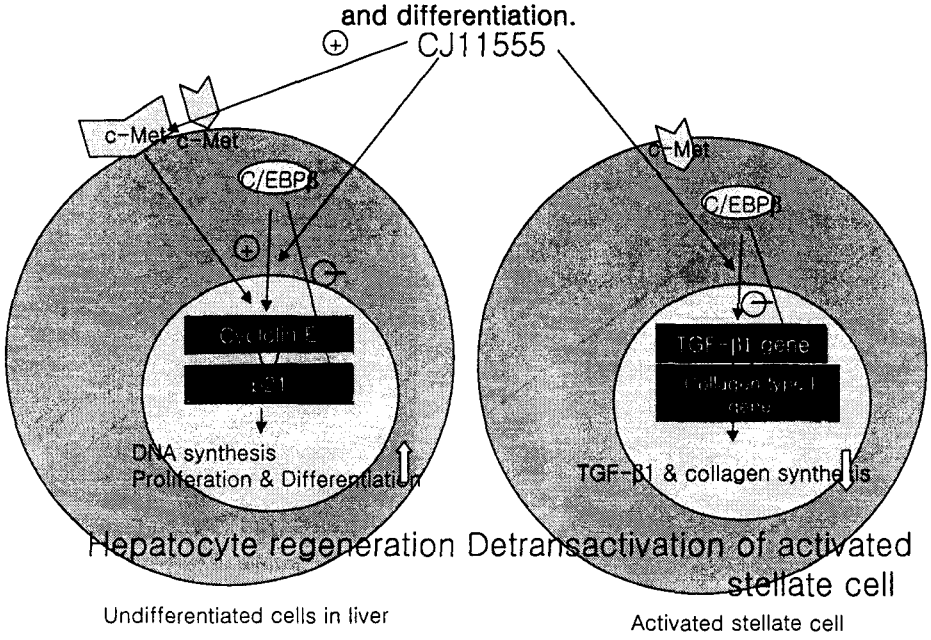




α-Smooth muscle actin immuno-histochemistry



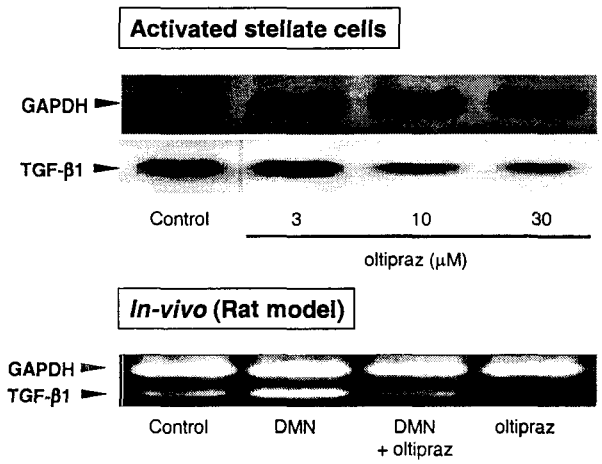
Oltipraz regenerates cirrhotic liver through C/EBP-mediated coordinate control of activated stellate cells and of hepatocyte growth and differentiation.

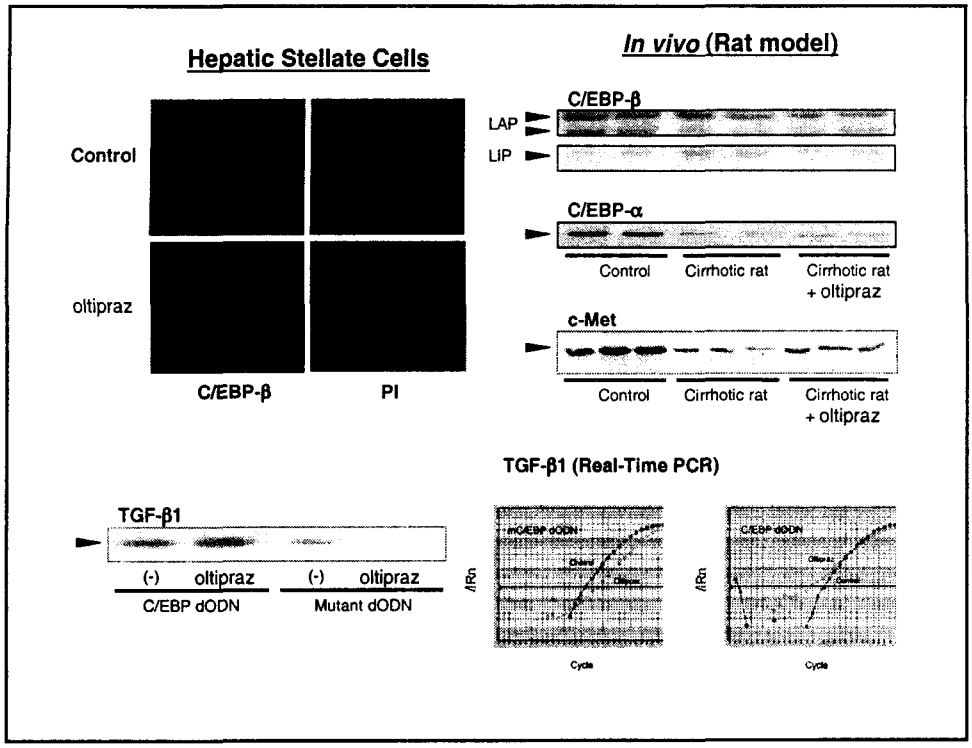


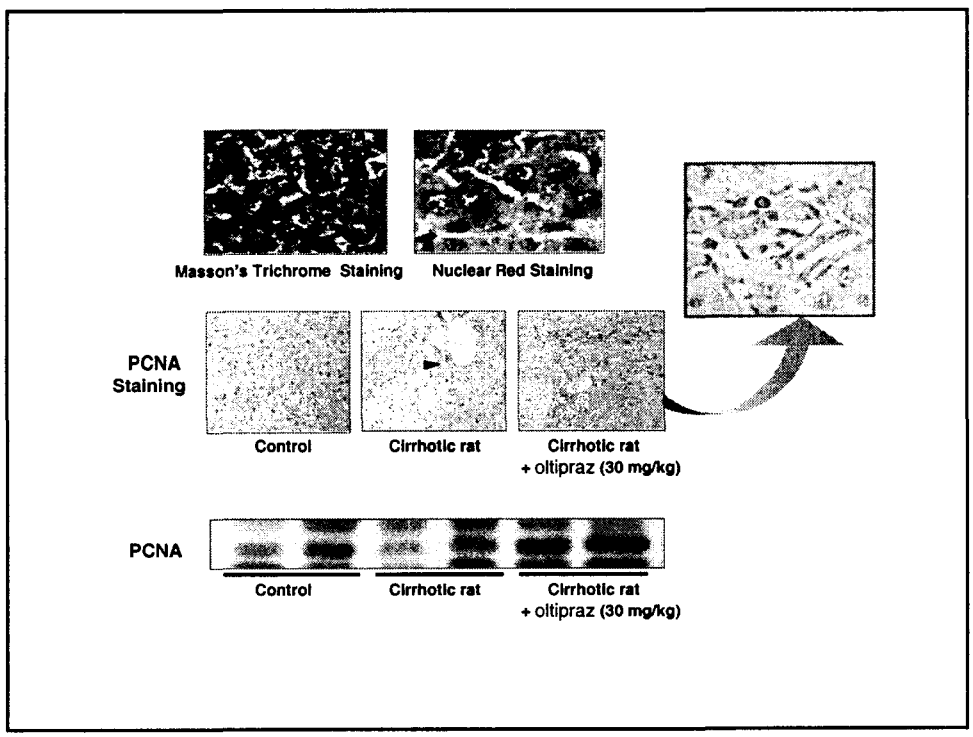
Hepatocyte regeneration Detransactivation of activated stellate cell

Undifferentiated cells in liver

Activated stellate cell

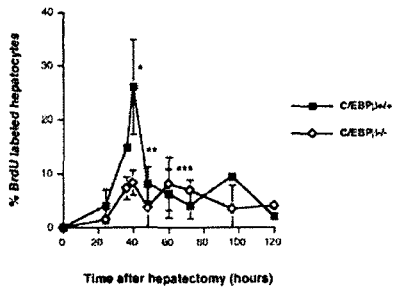



Hepatic Stellate Cells
In vivo (Rat model)
Control
oltipraz
C/EBP-β
PI
C/EBP-β
LAP
LIP
C/EBP-α
Control
Cirrhotic rat
Cirrhotic rat + oltipraz
c-Met
Control
Cirrhotic rat
Cirrhotic rat + oltipraz
TGF-β1 (Real-Time PCR)
TGF-β1
(-) oltipraz
(-) oltipraz
C/EBP dODN
Mutant dODN
ΔRn
Cycle

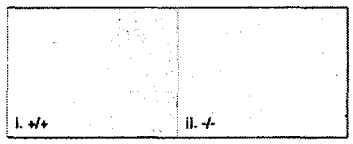

Masson's Trichrome Staining
Nuclear Red Staining
PCNA Staining
Control
Cirrhotic rat
Cirrhotic rat + oltipraz (30 mg/kg)
PCNA
Control
Cirrhotic rat
Cirrhotic rat + oltipraz (30 mg/kg)

C/EBPβ is necessary for the regeneration of hepatocytes after partial hepatecvtomy

A



B

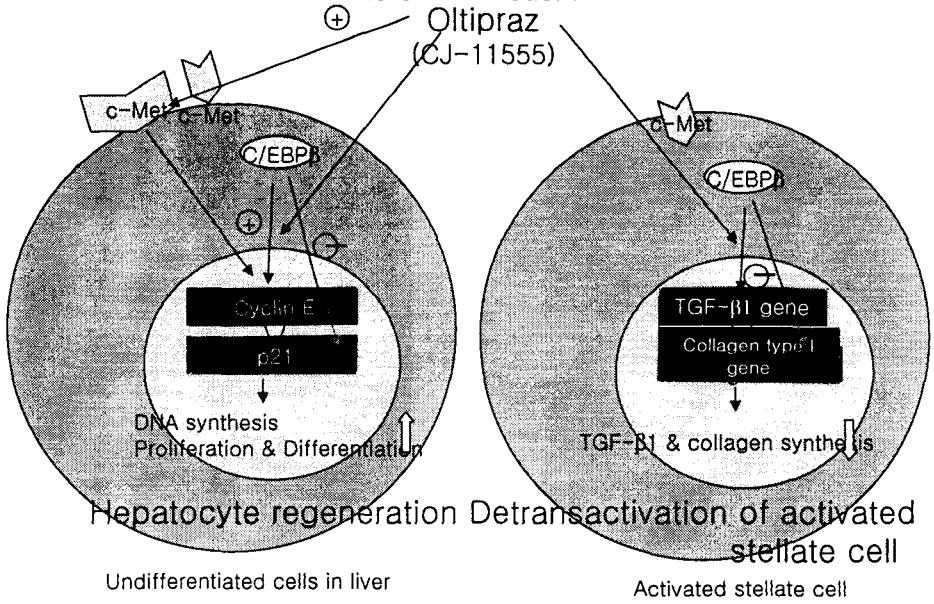


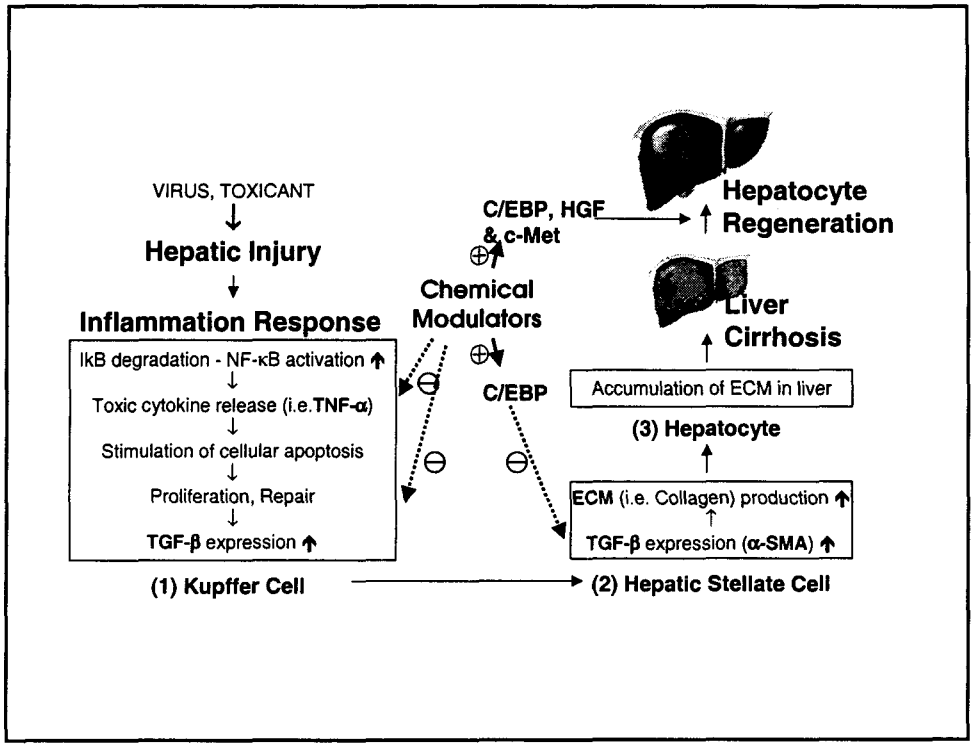
In C/EBPβ knock-out mice

1) Decrease in S phase hepatocyte
2) Decrease in **cyclin** E & cyclin B expression

Greenbaum et al., C/EBPβ is required for normal hepatocyte proliferation in mice after partial hepatectomy. J Clin Invest. 1998 102:996-1007.

Oltipraz regenerates cirrhotic liver through C/EBP-mediated coordinate control of activated stellate cells and of hepatocyte growth and differentiation.




VIRUS, TOXICANT
Hepatic Injury
Inflammation Response
IkB degradation - NF-κB activation
Toxic cytokine release (i.e.TNF-α)
Stimulation of cellular apoptosis
Proliferation, Repair
TGF-β expression
(1) Kupffer Cell
Chemical Modulators
C/EBP, HGF & c-Met
C/EBP
Hepatocyte Regeneration
Liver Cirrhosis
Accumulation of ECM in liver
(3) Hepatocyte
ECM (i.e. Collagen) production
TGF-β expression (α-SMA)
(2) Hepatic Stellate Cell