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Review

The hepatic stellate cell in the post-genomic era

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Summary. The draft human genome sequence was published on February 15, 2001, which will provide a huge amount of information on human genetics, human disease, and human cell biology. Now, medical scientists and cell biologists are turning their attention to illustrating gene expression pattern using gene microarray and to identifying the functions and the expression patterns of proteins encoded by the genes.

Hepatic stellate cell is one of the sinusoid-constituent cells that play multiple roles in the liver pathophysiology. Transformation of stellate cells from the vitamin A-storing phenotype to the "myofibroblastic" one closely correlates to hepatic fibrosis during chronic liver trauma. Analyses of the molecular mechanisms of stellate cell activation have made a great progress, in particular, in the field of intracellular signal transduction of transforming growth factor-ß and platelet-derived growth factor, integrin signaling related to cell-adhesion, and cell motility-associated Rho and focal-adhesion kinase. Accumulation of the information on the stellate cell activation would shed light on the establishment of a novel therapeutic strategy against fibrosis of human liver disease.

Key words: Stellate cell, Liver fibrosis, Genomics, Proteomics

Progress in the molecular analysis of stellate cell activation

Signaling and transcriptional regulation of collagen gene expression

Transforming growth factor-ß1 (TGFß1) is the most important fibrogenic cytokine in hepatic fibrosis (Friedman, 1999). Cellular sources of TGFß1 are multiple, including hepatic stellate cell, Kupffer cell, hepatocyte, sinusoidal endothelial cell and platelet. Proteolytic cleavage of latent TGF-ß binding protein (LTBP) is supposed to be a prerequisite for the release and generation of bioactive (mature) TGF-ß, which is induced by urokinase plasminogen activator (uPA) or tissue PA (tPA) (Yee et al., 1993). Kojima et al. (2000) reported that Zf9 transcriptionally activates uPA, resulting in the increase of bioactive TGF-ß. Okuno et al. (1997) reported that retinoids exacerbate rat liver fibrosis by inducing the activation of latent TGF-ß in stellate cells. In vitro, 9-cis-retinoic acid enhanced cellular PA and plasmin levels thereby inducing plasmin-mediated activation of latent TGF-ß (Imai et al., 1997).

TGF-ß regulates gene expression through activating two kinds of receptor-associated serine/threonine protein kinases and through phosphorylating receptor-regulated Smad2 and Smad3, allowing them to associate with Smad4 and to translocate into the nucleus (Wells, 2000). In the nucleus, the Smad complex associates with a DNA-binding partner, such as Fast-1, and this complex binds to specific enhancers in the target genes, resulting in the activation of transcription (Zhou et al., 1998).

In terms of transcriptional regulation of collagen gene expression, there are three protein binding sites, 5A, 3A and B, in the alpha 2(I) collagen gene (COL1A2) promoter region (Inagaki et al., 1994). C/EBPß, Sp-1, and Smad3 bind to 5A, 3A and B regions, respectively, of TGF-beta-responsive element (TbRE). The interaction between Sp1 and Smad 3 plays the essential role for basal and TGF-ß-stimulated transcription of COL1A2 in stellate cell and skin fibroblast (Zhang et al., 2000). Stefanovic et al. (1997) reported that 3' UTR of collagen α1(I) mRNA binds alpha complex protein (CP), a protein implicated in increasing stability of collagen α1(I) mRNA in activated stellate cell. Collagen α1(I) mRNA is regulated by a complex interaction between the 5' stem-loop and the 3' UTR.

Regulation of ECM turnover by MMPs and TIMPs

Knittel et al. (1999) clarified the cellular origin of metalloproteinases (MMPs)/tissue inhibitor of matrix metalloproteinases (TIMPs) within the liver. MMP-2

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(gelatinase-A) expression was prominent in stellate cells and myofibroblasts prepared from rat liver. MMP-9 (gelatinase-B) was detected in myofibroblasts, Kupffer cell, stellate cell and hepatocyte. MMP-3 (stromelysin-1), MMP-10 (stromelysin-2) and MMP-13 (collagenase) were prominent in stellate cell. MMP-14 (membrane type-1 MMP) expression was present in both parenchymal and nonparenchymal liver cells. TIMP-1 was predominantly present in stellate cells and myofibroblasts, while TIMP-2 was additionally expressed by Kupffer cell. In contrast, TIMP-3 was detectable in hepatocyte only in minor quantities. Their activity is regulated by several mechanisms, including the regulation of gene expression by cytokines or hormones, and extracellular cleavage of the proenzyme to form the active enzyme by TIMPs.

Okazaki et al. (2000) emphasizes that interstitial collagenases are key enzymes for ameliorating liver fibrosis. They also reported that MMP-13 was detected strikingly under the recovery phase of liver fibrosis (Watanabe et al., 2000). In contrast, Arthur and Iredale emphasized that the regression of liver fibrosis is mediated by the decreased expression of TIMPs, demonstrating that mRNA level of TIMPs decreased gradually in the rat liver after stopping injection of CCl_4, while that of interstitial collagenases (MMP-1 and MMP-13) remained unchanged (Iredale et al., 1998; McCrudden and Iredale, 2000). By using a liver-targeted TIMP-1 transgenic mouse under the control of the albumin promoter/enhancer, Yoshiji et al. (2000) concluded that TIMP-1 strongly promotes the development of liver fibrosis.

Among other types of MMPs, Takahara et al. (1997) reported that dual expression of MMP-2 and MT1-MMP were detected in chronic hepatitis and cirrhosis. They suggested that MT1-MMP activates pro-MMP-2 and the activated MMP-2 may remodel liver parenchyma during the process of liver fibrosis.

Proliferation-related signaling in stellate cells

Platelet-derived growth factor (PDGF) is a most potent mitogen for stellate cell as well as other certain cell types (Pinzani et al., 1989). PDGF is a dimeric molecule consisting of disulfide-bonded, structurally similar A- and B-polypeptide chains, which combine to homo- and hetero-dimers. The PDGF isoforms exert their cellular effects by binding to and activating two structurally related protein tyrosine kinase receptors, denoted the α-receptor and the ß-receptor. Activation of PDGF receptors leads to the stimulation of cell growth, and also to the change in cell shape and motility.

The autophosphorylation induced after dimerization of PDGF receptors activates transduction molecules containing SH2 domains, such as phosphatidylinositol 3-kinase (PI3-kinase), phospholipase C (PLC)-γ, the Src family of tyrosine kinases, the tyrosine phosphatase SHP-2, and a GTPase activating protein (GAP) for Ras (Heldin et al., 1998).

In human stellate cell, activation of the extracellular-signal regulated kinase (ERK) pathway followed by an increased expression of c-fos in response to PDGF has been demonstrated in some studies (Parola et al., 1998). Agents that are able to elevate intracellular cAMP levels may reduce cell growth via inhibiting Raf kinase, an upstream activator of ERK, through phosphorylation of Raf-1 by cAMP-activated protein-kinase (PKA). Marra et al. (1999) reported that binding of the AP-1 complex and of STAT to the related regulatory elements was mediated by ERK. Reeves et al. (2000) also reported that the potent mitogenic effect of PDGF in stellate cells might be caused, in part, by the generation of lipid-derived second messenger phosphatidic acid (PA) and subsequently by a more sustained activation of ERK in stellate cell.

Members of the PI3-kinase family that binds to and are activated by tyrosine kinase receptors consist of a regulatory subunit, p85, and a catalytic subunit, p110. The downstream effectors of PI3-Kinase include protein kinase Cz, ribosomal S6 kinase, and protein kinase B (c-Akt). This pathway has been shown to be sufficient to transduce PDGF-dependent mitogenic signals and to be necessary for cell chemotaxis (Marra et al., 1997).

Phospholipase C-γ mobilizes intracellular Ca^{2+} from internal stores and activates certain members of the PKC family. Carloni et al. (1997) reported that PDGF-BB stimulated stellate cell migration through FAK phosphorylation associated with PLC-γ tyrosine phosphorylation.

The family of STAT molecules has seven members, of which STAT1, STAT3, STAT5a, STAT5b and STAT6 have been shown to bind to the activated PDGF receptor-ß and to be phosphorylated after PDGF stimulation. After phosphorylation on tyrosine residues, STATs dimerize and translocate to the nucleus, where they act as the transcription factors. In stellate cell, dibutyryl cAMP attenuated STAT1 activation in HSC stimulated with PDGF-BB (Kawada et al., 1997).

A negative feedback mechanism involves cAMP-dependent protein kinase, which is activated by PDGF through the induction of prostaglandin synthesis and activation of adenylyl cyclase. Mallat et al. (1998) reported that PDGF-BB and thrombin generate growth inhibitory prostaglandin E2 and cAMP through promoting delayed induction of cyclooxygenase (COX)-2 in human stellate cell.

The Na^+/H^+ exchanger is the main intracellular pH regulator in stellate cell and its activity is increased by PDGF (Di Sario et al., 1999). PDGF induced a significant increase in the Na^+/H^+ exchanger activity through calcium/calmodulin- and protein kinase C-dependent pathways, leading to the proliferation of stellate cell (Benedetti et al., 2001).

Peroxisome proliferator-activated receptor gamma (PPARγ) regulates adipocyte differentiation and controls gene transcription in response to various activators (Kubota et al., 1999). Galli et al. (2000) showed that the depression of PPARγ expression and activity is involved

in the proliferation of stellate cell and that the activation of ligand-mediated PPARγ exerts an inhibition of PDGF-induced mitogenesis in activated human stellate cell. Miyahara et al. (2000) also reported that stellate cell activation is associated with the reduction of PPARγ expression. In addition, Marra et al. (2000) reported that PPARγ agonists also inhibited the chemotaxis of stellate cell induced by PDGF.

Signals controlling stellate cell contraction

At tissue level, the contractile force generated by stellate cells may induce scar constriction in cirrhosis and restricts sinusoidal blood flow. Agents, such as endothelin-1 (ET-1), angiotensin II, and vasopressin, can induce stellate cell contraction (Bataller et al., 2000). Recent reports have shown that L-type Ca^{2+} channel expression is up-regulated in stellate cells that are activated *in vivo* as well as *in vitro*, and that these channels modulate both Ca^{2+} signaling and contractile force generation (Bataller et al., 2001).

RhoA/rho-associated kinase signaling pathway is a mediator of contraction in muscle cells. Activation of Rho induces phosphatidylinositol-4,5-bisphosphate (PIP_2) production and stimulates ROCK and Dia by disrupting intramolecular inhibitory interactions (Watanabe et al., 1999). ROCK controls actomyosin filament assembly and myosin contractile activity by increased phosphorylation of the myosin light chain (MLC) (Totsukawa et al., 2000). Recently, the ras-like GTPase, rhoA, has been investigated as a mediator of stellate cell contraction (Yee, 2001). Kawada et al. (1999) reported that the contraction of stellate cells induced by ET-1 was attenuated by Y-27632, an inhibitor of Rho-associated kinase. Iwamoto et al. (2000) reported that Y-27632 inhibited the phosphorylation of focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK) and that stellate cells treated with Y-27632 failed to proliferate. Nishi et al. (1999) showed that RhoN, a novel small GTP-binding protein, was predominantly expressed in neurons and hepatic stellate cells. Yanase et al. (2000) demonstrated the involvement of RhoA in lysophosphatidic acid-induced signalings.

ETs are important regulators of the hepatic microcirculation. ET-1 is well known to induce contraction of cultured stellate cells on hydrated collagen gels (Kawada et al., 1993). ET-1 synthesis is regulated by endothelin-converting enzyme-1 (ECE-1) during hepatic wound healing. Shao et al. (1999) reported that ET-1 release is increased in stellate cells, whereas markedly decreased in endothelial cells after liver injury, depending on the ECE-1 mRNA expression and its stability.

Expression of ET receptors is regulated by TGF-ß1, reactive oxygen species, and endotoxin. TGF-ß1 decreased ET-1 receptor density through reduction of ET-1 mRNA expression (Gabriel et al., 1999). Oxidative stress induced by hypoxanthine/xanthine oxidase caused an initial decrease in ET-1 receptor, followed by a significant increase over the basal level (Gabriel et al., 1998). Endotoxin causes up-regulation of ET receptors in cultured stellate cells via nitric oxide (NO)-dependent and -independent mechanisms (Bauer et al., 2000).

NO is an additionally potent mediator of sinusoidal hemodynamics. Kawada et al. (1998a) reported that NO-dependent increase of cellular cGMP level mediates the inhibitory effect of LPS and IFNγ on the expression of smooth muscle-α actin in stellate cells. Failli et al. (2000) showed that NO donors, such as nitroglycerin (NTG) and S-nitroso-N-acetyl penicillamine (SNAP), induced a dose-dependent decrease in PDGF-triggered DNA synthesis and cell migration, which was associated with the attenuation of PDGF-induced increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$) and activation of ERK. Yu et al. (2000) reported that overexpression of neuronal NOS inhibited ET-1-induced contraction of stellate cell.

Gorbig et al. (2001) reported that cultured human stellate cells have receptors for a vasodilator peptide adrenomedullin. Stimulation of stellate cell with adrenomedullin resulted in a dose-dependent rise in cAMP concentration and markedly blunted the endothelin-induced increase of $[Ca^{2+}]_i$ and cell contraction. The existence of a receptor CRLR for adrenomedullin and the associated-proteins, RAMP-1 and RAMP-2, was demonstrated (Nagae et al., 2000). Human stellate cells secreted adrenomedullin in the culture medium, which was markedly enhanced by TNF-α and IL-1ß. These results indicated that adrenomedullin can regulate stellate cell contractility in an autocrine manner. In addition, atrial natriuretic peptide (ANP) regulates stellate cell contraction induced by ET-1 (Gorbig et al., 1999).

Cell-adhesion-related signaling

The integrin family of cell surface receptors, heterodimers composed of α and ß transmembrane subunits linked noncovalently, has been shown to play an important role in mediating the interaction between cells and ECM and regulating several cell functions, including motility. Stellate cell expresses integrin $\alpha_1ß_1$, $\alpha_2ß_1$, $\alpha_vß_1$ and $\alpha_6ß_4$ (Imai et al., 2000). Iwamoto et al. (1998) showed that inhibition of integrin signaling using RGD motifs diminished the adhesion-induced tyrosine phosphorylation of FAK, and inhibited the formation of stress fibers and the expression of smooth muscle α-actin in stellate cell. Integrin antagonism using GRGDS peptide induced apoptosis of stellate cell through increasing p53 and decreasing the Bcl-2/Bax ratio (Iwamoto et al., 1999). Kato et al. (2001) identified the anti-adhesive site buried within the heparin-binding domain of fibronectin, YTIVIAL sequence in the 14th type III module, which down-regulated the myofibroblastic conversion of stellate cell.

Several proteins present in the focal adhesion complex have been shown to undergo tyrosine phosphorylation. One protein is the p125FAK, a 125-kilodalton non-receptor tyrosine kinase (Sieg et al.,

1999). FAK, in conjunction with paxillin, plays an important role in localizing several Src homology 2 (SH2) domain-containing proteins, such as Src family member kinases, adapter protein Grb2, and PI3-Kinase (Qiu and Kung, 2000). PLCγ is able to associate with FAK (Zhang et al., 1999). Pinzani et al. (1998) reported that the interaction between FAK and PLCγ was involved not only in integrin-mediated signaling pathways but also in PDGF-BB-dependent migration of stellate cell.

The discoidin domain receptor 2 (DDR2) has been cloned from activated stellate cells and interacts with fibrillar collagens (Ankoma-Sey et al., 1998). The identification of DDR2 mRNA and protein in activated stellate cells suggests that it may be a key receptor in hepatic fibrosis. Because fibrillar collagens are produced by activated stellate cells, DDR2 receptor signals as an autocrine stimulus.

Signaling of inflammatory cytokines and the role of NF-κB

Nuclear factor-κB (NF-κB) has been the most extensively studied transcription factor in stellate cells. The NF-κB family of transcription factors is related by their Rel homology domain (RHD) and comprises at least five members, including p65 (RelA), p50, p52, RelB, and c-Rel, that form homo- or hetero-dimers. The NF-κB dimers p65:p65 and p50:p65 have been identified in stellate cells (Vasiliou et al., 2000). Hellerbrand et al. (1998) reported that activation of NF-κB induced by TNF-α and IL-1ß leads to the expression of ICAM-1, IL-6, and macrophage inflammatory protein-2 in rat stellate cell. Gallois et al. (1998) reported that activation of NF-κB by ET-1 and TNF-α leads to increased COX-2. Rippe et al. (1999) reported that NF-κB activation by TNF-α also inhibits expression of the α1(I) collagen gene. However, Lang et al. (2000) pointed out that NF-κB activity is not necessarily required for stellate cell activation, such as proliferation and collagen production.

Therapeutic strategy for preventing liver fibrosis

Increasing numbers of studies have shown a variety of therapeutic approaches for liver fibrosis based on the molecular inhibition of stellate cell activation. Among them, IFN has a clinical potential to treat liver fibrosis by eliminating HCV virus from patients (Shiratori et al., 2000). Rockey and Chung (1994) reported that IFNγ inhibits stellate cell activation and collagen type I mRNA expression in rats. Higashi et al. (1998) showed that IFNγ inhibits the collagen transcription through directly acting onto IFN-responsive element, a proximal element within the human α2(I) collagen (COL1A2) promoter. Ulloa et al. (1999) reported that IFNγ inhibits TGF-ß/Smad signaling through STAT pathway.

In animal models, the blockade of TGF-ß signaling has a potential to suppress liver fibrosis. George et al. (1999) reported that liver fibrosis could be inhibited by a soluble receptor consisting of a chimeric IgG at the extracellular portion of the TGF-ß type II receptor. Qi et al. (1999) reported that liver fibrosis was suppressed through the inhibition of TGF-ß signaling by the adenovirus-mediated local expression of a dominant-negative type II TGF-ß receptor (AdCATß-TR). Moreover, Nakamura et al. (2000) reported that a soluble TGF-ß receptor overexpression by injecting AdTß-EXR into muscle prevents liver fibrogenesis in rats.

Pinzani et al. (1996) reported that pentoxifylline inhibited the PDGF-dependent activation of ERK in stellate cells. Kawada et al. (1998b) reported that antioxidants, such as resveratrol, quercetin and N-acetylcysteine, dose-dependently suppressed serum-dependent proliferation of stellate cells via the suppression of inositol phosphate metabolism, tyrosine phosphorylation, and the activation of MAP kinase in PDGF/BB-stimulated stellate cells. Wang et al. (2000) reported that the semisynthetic analogue of fumagillin TNP-470 inhibited HSC proliferation by blocking the cell-cycle transition from G1 to S in the absence and/or presence of PDGF, and, as the consequence, prevented the progression of hepatic fibrosis. Zhu et al. (1999) reported that a new immunosuppressive agent, rapamycin, inhibited extracellular matrix deposition in the rat fibrosis model and decreased PDGF-induced proliferation of stellate cells. Benedetti et al. (2001) reported that the Na^+/H^+ exchanger inhibitor 5-N-ethyl-N-isopropyl-amiloride inhibited all the effects derived by PDGF, FeAsc, and FeNTA. In vivo, amiloride reduced HSC proliferation, activation, collagen deposition, and collagen synthesis.

Recently, we found that N-acetylcysteine triggers the degradation of PDGF receptor ß mediated by cathepsin B (Okuyama et al., 2001). N-acetylcysteine inhibits PDGF signaling, PDGF-dependent DNA synthesis, and in addition, affected the expression of TGF-ß receptor type II. N-acetylcysteine dramatically attenuated liver fibrosis (Fig. 1).

HGF gene therapy is promising for the treatment of liver fibrosis. Ueki et al. (1999) reported that repeated transfections of the human HGF gene into skeletal muscle suppressed the increase of TGF-ß1, inhibited fibrogenesis and hepatocyte apoptosis, and resolved completely fibrosis in the cirrhotic liver. Sato et al. (2000) reported that continuous administration of recombinant human HGF (rhHGF) was also effective for liver fibrosis. Although rhHGF reduced mRNA levels of procollagen α2(I), α1(IV) and TGF-ß1, the detailed molecular mechanism of these anti-fibrotic effects of HGF remains to be elucidated.

Telomerase gene therapy was reported to inhibit liver cirrhosis in mice. Accelerated telomere loss has been proposed to be a factor leading to end-stage organ failure in chronic diseases of high cellular turnover such as liver cirrhosis. Rudolph et al. (2000) showed that adenoviral delivery of telomerase RNA gene into the livers of telomerase-deficient mice with short

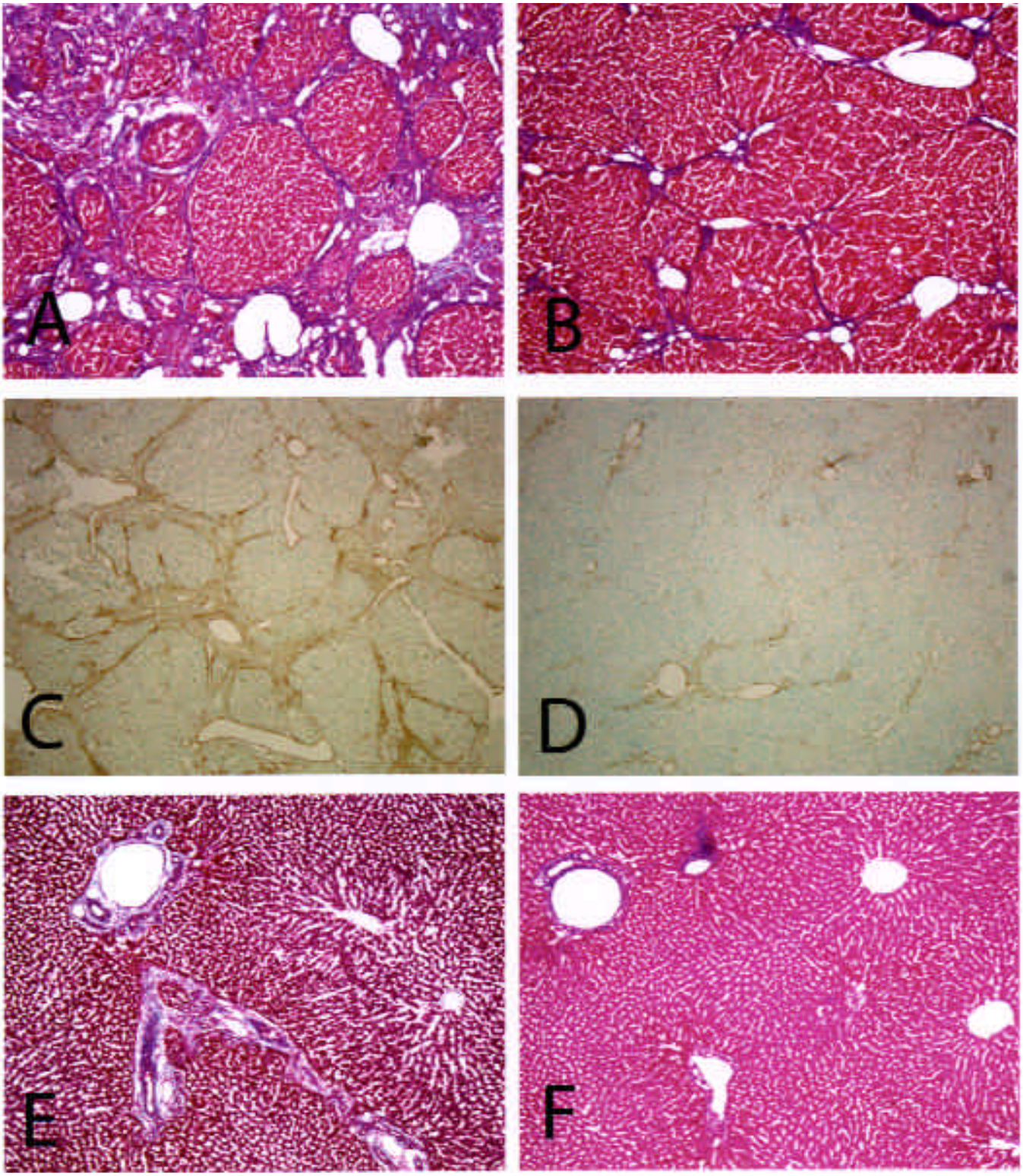

Fig. 1. Effect of N-acetylcysteine on toxin- or bile-duct ligation-induced liver fibrosis. **A.** Thioacetamide (TAA)-induced liver fibrosis. TAA was administered at 50 mg/rat twice a week for 6 weeks. Note the development of liver fibrosis with thick bundles of septum. Azan staining, x 100. **B.** TAA-induced liver fibrosis treated with N-acetylcysteine (NAC). NAC was administered intraperitoneally at 100 mg/rat every day for 6 weeks together with TAA injection. Degree of fibrosis was suppressed. Azan staining, x 100. **C.** Immunostaining of smooth muscle α-actin in TAA-induced liver fibrosis. Smooth muscle α-actin-positive cells were numerous along the septum of fibrosis. x 100. **D.** Immunostaining of SM α-actin in TAA-induced liver fibrosis treated with NAC. The number of smooth muscle α-actin-positive cells was dramatically decreased. x 100. **E.** Bile duct ligation (BDL)-induced liver fibrosis Two weeks after bile duct ligation was performed under laparotomy, rats were sacrificed. Peri-ductular fibrosis was developed around portal vein area. Azan staining, x 100. **F.** BDL-induced liver fibrosis treated with NAC. NAC was administered intraperitoneally at 100 mg/rat every day for 2 weeks. Note that liver parenchyma exhibits almost intact architecture after NAC treatment. Azan staining, x 100

dysfunctional telomeres restored telomerase activity and telomere function, alleviated cirrhotic pathology, and improved liver function.

A new approach for the molecular analysis of stellate cell activation

Discovery of genes associated with stellate cell activation

Subtractive hybridization has been utilized to clone genes associated with the activation of stellate cells. Friedman et al. cloned a novel Kruppel-like factor 6 (KLF6)/Zf9/COPEB/GBF as an immediate-early gene induced in stellate cells after acute liver injury (Ratziu et al., 1998). This zinc finger transcription factor has a number of potential transcriptional targets including collagen α1(I), TGF-ß1 and its receptors, as well as urokinase type plasminogen activator (uPA) (Kojima et al., 2000). Recently, they reported that this factor has an antiproliferative activity (Kim et al., 1998).

By using suppression subtractive hybridization, Ikeda et al. (1998) identified 13 genes dominantly expressed in activated stellate cells, one of which was identical to rat prion-related protein (PrP). They showed that PrP expression was closely related to stellate cell activation associated with liver fibrosis. Kitada et al. (2000) reported that PrP is utilized as a marker of activated stellate cells in human chronic liver diseases.

Although still not applied to stellate cell, the gene microarray method will have more potential to elucidate the genes associated with the cell activation and liver fibrosis. These approaches promise to revolutionize the way to create new paradigms of regulation of liver fibrosis that are not even conceivable based on current knowledge.

Proteomics

In 2000, Kristensen et al. (2000) applied the proteomics to the analysis of stellate cell activation for the first time. They performed 2-D SDS-PAGE analysis on cellular and secreted proteins of quiescent and activated stellate cells, with a special emphasis on proteins displaying activation-associated changes in their expression levels. They showed that a total of 43 proteins/polypeptides altered their expression levels when the cells were activated in vivo and/or in vitro. Three growth- and proliferation-associated proteins, calcyclin, calgizzarin, and galectin-1, become heavily up-regulated in the activated stellate cells, representing a new finding.

Kawada et al. (2001) cloned a novel protein named STAP as a stellate cell activation-associated protein by using this proteome procedure. STAP is a cytoplasmic protein with molecular weight of 21,496 and shows about 40% amino acid sequence homology with myoglobin. STAP is dramatically induced in *in vivo* activated stellate cells isolated from fibrotic liver and in stellate cells undergoing in vitro activation during primary culture.

In summary, molecular analysis of stellate cell has uncovered the cell-specific events closely associated with the cell activation, deeply contributing to the development or the resolution of liver fibrosis. Application of modern technology, such genomics and proteomics, to the study will be required for the establishment of therapeutic strategy for liver.

References

Ankoma-Sey V., Matli M., Chang K.B., Lalazar A., Donner D.B., Wong L., Warren R.S. and Friedman S.L. (1998). Coordinated induction of VEGF receptors in mesenchymal cell types during rat hepatic wound healing. Oncogene 17, 115-121.

Bataller R., Gines P., Nicolas J.M., Gorbig M.N., Garcia-Ramallo E., Gasull X., Bosch J., Arroyo V. and Rodes J. (2000). Angiotensin II induces contraction and proliferation of human hepatic stellate cells. Gastroenterology 118, 1149-1156.

Bataller R., Gasull X., Gines P., Hellemans K., Gorbig M.N., Nicolas J.M., Sancho-Bru P., De Las Heras D., Gual A., Geerts A., Arroyo V. and Rodes J. (2001). In vitro and in vivo activation of rat hepatic stellate cells results in de novo expression of L-type voltage-operated calcium channels. Hepatology 33, 956-962.

Bauer M., Bauer I., Sonin N.V., Kresge N., Baveja R., Yokoyama Y., Harding D., Zhang J.X. and Clemens M.G. (2000). Functional significance of endothelin B receptors in mediating sinusoidal and extrasinusoidal effects of endothelins in the intact rat liver. Hepatology 31, 937-947.

Benedetti A., Di Sario A., Casini A., Ridolfi F., Bendia E., Pigini P., Tonnini C., D'Ambrosio L., Feliciangeli G., Macarri G. and Svegliati-Baroni G. (2001). Inhibition of the NA(+)/H(+) exchanger reduces rat hepatic stellate cell activity and liver fibrosis: an in vitro and in vivo study. Gastroenterology 120, 545-556.

Carloni V., Romanelli R.G., Pinzani M., Laffi G. and Gentilini P. (1997). Focal adhesion kinase and phospholipase C gamma involvement in adhesion and migration of human hepatic stellate cells. Gastroenterology 112, 522-531.

Di Sario A., Bendia E., Svegliati Baroni G., Ridolfi F., Bolognini L., Feliciangeli G., Jezequel A.M., Orlandi F. and Benedetti A. (1999). Intracellular pathways mediating Na^+/H^+ exchange activation by platelet-derived growth factor in rat hepatic stellate cells. Gastroenterology 116, 1155-1166.

Failli P., DeFranco R.M., Caligiuri A., Gentilini A., Romanelli R.G., Marra F., Batignani G., Guerra C.T., Laffi G., Gentilini P. and Pinzani M. (2000). Nitrovasodilators inhibit platelet-derived growth factor-induced proliferation and migration of activated human hepatic stellate cells. Gastroenterology 119, 479-492.

Friedman S.L. (1999). Cytokines and fibrogenesis. Semin. Liver Dis. 19, 129-140.

Gabriel A., Kuddus R.H., Rao A.S., Watkins W.D. and Gandhi C.R. (1998). Superoxide-induced changes in endothelin (ET) receptors in hepatic stellate cells. J. Hepatol. 29, 614-627.

Gabriel A., Kuddus R.H., Rao A.S. and Gandhi C.R. (1999). Down-regulation of endothelin receptors by transforming growth factor beta1 in hepatic stellate cells. J. Hepatol. 30, 440-450.

Galli A., Crabb D., Price D., Ceni E., Salzano R., Surrenti C. and Casini A. (2000). Peroxisome proliferator-activated receptor gamma

transcriptional regulation is involved in platelet-derived growth factor-induced proliferation of human hepatic stellate cells. Hepatology 31, 101-108.

Gallois C., Habib A., Tao J., Moulin S., Maclouf J., Mallat A. and Lotersztajn S. (1998). Role of NF-kappaB in the antiproliferative effect of endothelin-1 and tumor necrosis factor-alpha in human hepatic stellate cells. Involvement of cyclooxygenase-2. J. Biol. Chem. 273, 23183-23190.

George J., Roulot D., Koteliansky V.E. and Bissell D.M. (1999). In vivo inhibition of rat stellate cell activation by soluble transforming growth factor beta type II receptor: a potential new therapy for hepatic fibrosis. Proc. Natl. Acad. Sci. USA 96, 12719-12724.

Gorbig M.N., Gines P., Bataller R., Nicolas J.M., Garcia-Ramallo E., Tobias E., Titos E., Rey M.J., Claria J., Arroyo V. and Rodes J. (1999). Atrial natriuretic peptide antagonizes endothelin-induced calcium increase and cell contraction in cultured human hepatic stellate cells. Hepatology 30, 501-509.

Gorbig M.N., Gines P., Bataller R., Nicolas J.M., Garcia-Ramallo E., Cejudo P., Sancho-Bru P., Jimenez W., Arroyo V. and Rodes J. (2001). Human hepatic stellate cells secrete adrenomedullin: potential autocrine factor in the regulation of cell contractility. J. Hepatol. 34, 222-229.

Heldin C.H., Ostman A. and Ronnstrand L. (1998). Signal transduction via platelet-derived growth factor receptors. Biochim. Biophys. Acta 1378, F79-F113.

Hellerbrand C., Jobin C., Licato L.L., Sartor R.B. and Brenner D.A. (1998). Cytokines induce NF-kappaB in activated but not in quiescent rat hepatic stellate cells. Am. J. Physiol. 275, G269-G278.

Higashi K., Kouba D.J., Song Y.J., Uitto J. and Mauviel A. (1998). A proximal element within the human alpha 2(I) collagen (COL1A2) promoter, distinct from the tumor necrosis factor-alpha response element, mediates transcriptional repression by interferon-gamma. Matrix Biol. 16, 447-456.

Ikeda K., Kawada N., Wang Y.Q., Kadoya H., Nakatani K., Sato M. and Kaneda K. (1998). Expression of cellular prion protein in activated hepatic stellate cells. Am. J. Pathol. 153, 1695-1700.

Imai K., Sato T. and Senoo H. (2000). Adhesion between cells and extracellular matrix with special reference to hepatic stellate cell adhesion to three-dimensional collagen fibers. Cell Struct. Funct. 25, 329-336.

Imai S., Okuno M., Moriwaki H., Muto Y., Murakami K., Shudo K., Suzuki Y. and Kojima S. (1997). 9,13-di-cis-Retinoic acid induces the production of tPA and activation of latent TGF-beta via RAR alpha in a human liver stellate cell line, LI90. FEBS Lett. 411, 102-106.

Inagaki Y., Truter S. and Ramirez F. (1994). Transforming growth factor-beta stimulates alpha 2(I) collagen gene expression through a cis-acting element that contains an Sp1-binding site. J. Biol. Chem. 269, 14828-14834.

Iredale J.P., Benyon R.C., Pickering J., McCullen M., Northrop M., Pawley S., Hovell C. and Arthur M.J. (1998). Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J. Clin. Invest. 102, 538-549.

Iwamoto H., Sakai H. and Nawata H. (1998). Inhibition of integrin signaling with Arg-Gly-Asp motifs in rat hepatic stellate cells. J. Hepatol. 29, 752-759.

Iwamoto H., Sakai H., Tada S., Nakamuta M. and Nawata H. (1999). Induction of apoptosis in rat hepatic stellate cells by disruption of integrin-mediated cell adhesion. J. Lab. Clin. Med. 134, 83-89.

Iwamoto H., Nakamuta M., Tada S., Sugimoto R., Enjoji M. and Nawata H. (2000). A p160ROCK-specific inhibitor, Y-27632, attenuates rat hepatic stellate cell growth. J. Hepatol. 32, 762-770.

Kato R., Kamiya S., Ueki M., Yajima H., Ishii T., Nakamura H., Katayama T. and Fukai F. (2001). The fibronectin-derived antiadhesive peptides suppress the myofibroblastic conversion of rat hepatic stellate cells. Exp. Cell Res. 265, 54-63.

Kawada N., Tran-Thi T.A., Klein H. and Decker K. (1993). The contraction of hepatic stellate (Ito) cells stimulated with vasoactive substances. Possible involvement of endothelin 1 and nitric oxide in the regulation of the sinusoidal tonus. Eur. J. Biochem. 213, 815-823.

Kawada N., Uoya M., Seki S., Kuroki T. and Kobayashi K. (1997). Regulation by cAMP of STAT1 activation in hepatic stellate cells. Biochem. Biophys. Res. Commun. 233, 464-469.

Kawada N., Seki S., Kuroki T. and Inoue M. (1998a). Regulation of stellate cell proliferation by lipopolysaccharide: role of endogenous nitric oxide. J. Gastroenterol. Hepatol. 13 (Suppl.), S6-S13.

Kawada N., Seki S., Inoue M. and Kuroki T. (1998b). Effect of antioxidants, resveratrol, quercetin, and N-acetylcysteine, on the functions of cultured rat hepatic stellate cells and Kupffer cells. Hepatology 27, 1265-1274.

Kawada N., Seki S., Kuroki T. and Kaneda K. (1999). ROCK inhibitor Y-27632 attenuates stellate cell contraction and portal pressure increase induced by endothelin-1. Biochem. Biophys. Res. Commun. 266, 296-300.

Kawada N., Kristensen D.B., Asahina K., Nakatani K., Minamiyama Y., Seki S. and Yoshizato K. (2001). Characterization of a stellate cell activation-associated protein (STAP) with peroxidase activity found in rat hepatic stellate cells. J. Biol. Chem. 276, 25318-25323.

Kim Y., Ratziu V., Choi S.G., Lalazar A., Theiss G., Dang Q., Kim S.J. and Friedman S.L. (1998). Transcriptional activation of transforming growth factor beta1 and its receptors by the Kruppel-like factor Zf9/core promoter-binding protein and Sp1. Potential mechanisms for autocrine fibrogenesis in response to injury. J. Biol. Chem. 273, 33750-33758.

Kitada T., Seki S., Ikeda K., Nakatani K., Sakaguchi H., Kawada N., Kadoya H. and Kaneda K. (2000). Clinicopathological characterization of prion: a novel marker of activated human hepatic stellate cells. J. Hepatol. 33, 751-757.

Knittel T., Mehde M., Kobold D., Saile B., Dinter C. and Ramadori G. (1999). Expression patterns of matrix metalloproteinases and their inhibitors in parenchymal and non-parenchymal cells of rat liver: regulation by TNF-alpha and TGF-beta1. J. Hepatol. 30, 48-60.

Kojima S., Hayashi S., Shimokado K., Suzuki Y., Shimada J., Crippa M.P. and Friedman S.L. (2000). Transcriptional activation of urokinase by the Kruppel-like factor Zf9/COPEB activates latent TGF-beta1 in vascular endothelial cells. Blood 95, 1309-1316.

Kristensen D.B., Kawada N., Imamura K., Miyamoto Y., Tateno C., Seki S., Kuroki T. and Yoshizato K. (2000). Proteome analysis of rat hepatic stellate cells. Hepatology 32, 268-277.

Kubota N., Terauchi Y., Miki H., Tamemoto H., Yamauchi T., Komeda K., Satoh S., Nakano R., Ishii C., Sugiyama T., Eto K., Tsubamoto Y., Okuno A., Murakami K., Sekihara H., Hasegawa G., Naito M., Toyoshima Y., Tanaka S., Shiota K., Kitamura T., Fujita T., Ezaki O., Aizawa S. and Kadowaki T. (1999). PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. Mol. Cell. 4, 597-609.

Lang A., Schoonhoven R., Tuvia S., Brenner D.A. and Rippe R.A. (2000). Nuclear factor kappaB in proliferation, activation, and apoptosis in rat hepatic stellate cells. J. Hepatol. 33, 49-58.

Mallat A., Gallois C., Tao J., Habib A., Maclouf J., Mavier P., Preaux A.M. and Lotersztajn S. (1998). Platelet-derived growth factor-BB and thrombin generate positive and negative signals for human hepatic stellate cell proliferation. Role of a prostaglandin/cyclic AMP pathway and cross-talk with endothelin receptors. J. Biol. Chem. 273, 27300-27305.

Marra F., Gentilini A., Pinzani M., Choudhury G.G., Parola M., Herbst H., Dianzani M.U., Laffi G., Abboud H.E. and Gentilini P. (1997). Phosphatidylinositol 3-kinase is required for platelet-derived growth factor's actions on hepatic stellate cells. Gastroenterology 112, 1297-1306.

Marra F., Arrighi M.C., Fazi M., Caligiuri A., Pinzani M., Romanelli R.G., Efsen E., Laffi G. and Gentilini P. (1999). Extracellular signal-regulated kinase activation differentially regulates platelet-derived growth factor's actions in hepatic stellate cells, and is induced by in vivo liver injury in the rat. Hepatology 30, 951-958.

Marra F., Efsen E., Romanelli R.G., Caligiuri A., Pastacaldi S., Batignani G., Bonacchi A., Caporale R., Laffi G., Pinzani M. and Gentilini P. (2000). Ligands of peroxisome proliferator-activated receptor gamma modulate profibrogenic and proinflammatory actions in hepatic stellate cells. Gastroenterology 119, 466-478.

McCrudden R. and Iredale J.P. (2000). Liver fibrosis, the hepatic stellate cell and tissue inhibitors of metalloproteinases. Histol. Histopathol. 15, 1159-1168.

Miyahara T., Schrum L., Rippe R., Xiong S., Yee H.F. Jr., Motomura K., Anania F.A., Willson T.M. and Tsukamoto H. (2000). Peroxisome proliferator-activated receptors and hepatic stellate cell activation. J. Biol. Chem. 275, 35715-35722.

Nagae T., Mukoyama M., Sugawara A., Mori K., Yahata K., Kasahara M., Suganami T., Makino H., Fujinaga Y., Yoshioka T., Tanaka I. and Nakao K. (2000). Rat receptor-activity-modifying proteins (RAMPs) for adrenomedullin/CGRP receptor: cloning and upregulation in obstructive nephropathy. Biochem. Biophys. Res. Commun. 270, 89-93.

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

Nishi M., Takeshima H., Houtani T., Nakagawara K., Noda T. and Sugimoto T. (1999). RhoN, a novel small GTP-binding protein expressed predominantly in neurons and hepatic stellate cells. Brain Res. Mol. Brain Res. 67, 74-81.

Okazaki I., Watanabe T., Hozawa S., Arai M. and Maruyama K. (2000). Molecular mechanism of the reversibility of hepatic fibrosis: with special reference to the role of matrix metalloproteinases. J. Gastroenterol. Hepatol. 15 (Suppl.), D26-D32.

Okuno M., Moriwaki H., Imai S., Muto Y., Kawada N., Suzuki Y. and Kojima S. (1997). Retinoids exacerbate rat liver fibrosis by inducing the activation of latent TGF-beta in liver stellate cells. Hepatology 26, 913-921.

Okuyama H., Shimahara Y., Kawada N., Seki S., Kristensen D.B., Yoshizato K., Uyama N. and Yamaoka Y. (2001). Regulation of cell growth by redox-mediated extracellular proteolysis of platelet-derived growth factor receptor beta. J. Biol. Chem. 276, 28274-28280.

Parola M., Robino G., Marra F., Pinzani M., Bellomo G., Leonarduzzi G., Chiarugi P., Camandola S., Poli G., Waeg G., Gentilini P. and Dianzani M.U. (1998). HNE interacts directly with JNK isoforms in human hepatic stellate cells. J. Clin. Invest. 102, 1942-1950.

Pinzani M., Gesualdo L., Sabbah G.M. and Abboud H.E. (1989). Effects of platelet-derived growth factor and other polypeptide mitogens on DNA synthesis and growth of cultured rat liver fat-storing cells. J. Clin. Invest. 84, 1786-1793.

Pinzani M., Marra F., Caligiuri A., DeFranco R., Gentilini A., Failli P. and Gentilini P. (1996). Inhibition by pentoxifylline of extracellular signal-regulated kinase activation by platelet-derived growth factor in hepatic stellate cells. Br. J. Pharmacol. 119, 1117-1124.

Pinzani M., Marra F. and Carloni V. (1998). Signal transduction in hepatic stellate cells. Liver 18, 2-13.

Qi Z., Atsuchi N., Ooshima A., Takeshita A. and Ueno H. (1999). Blockade of type beta transforming growth factor signaling prevents liver fibrosis and dysfunction in the rat. Proc. Natl. Acad. Sci. USA 96, 2345-2349.

Qiu Y. and Kung H.J. (2000). Signaling network of the Btk family kinases. Oncogene 19, 5651-5661.

Ratziu V., Lalazar A., Wong L., Dang Q., Collins C., Shaulian E., Jensen S. and Friedman S.L. (1998). Zf9, a Kruppel-like transcription factor up-regulated in vivo during early hepatic fibrosis. Proc. Natl. Acad. Sci. USA 95, 9500-9505.

Reeves H.L., Thompson M.G., Dack C.L., Burt A.D. and Day C.P. (2000). The role of phosphatidic acid in platelet-derived growth factor-induced proliferation of rat hepatic stellate cells. Hepatology. 31, 95-100.

Rippe R.A., Schrum L.W., Stefanovic B., Solis-Herruzo J.A. and Brenner D.A. (1999). NF-kappaB inhibits expression of the alpha1(I) collagen gene. DNA Cell. Biol. 18, 751-761.

Rockey D.C. and Chung J.J. (1994). Interferon gamma inhibits lipocyte activation and extracellular matrix mRNA expression during experimental liver injury: implications for treatment of hepatic fibrosis. J. Invest. Med. 42, 660-670.

Rudolph K.L., Chang S., Millard M., Schreiber-Agus N. and DePinho R.A. (2000). Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. Science 287, 1253-1258.

Shao R., Yan W. and Rockey D.C. (1999). Regulation of endothelin-1 synthesis by endothelin-converting enzyme-1 during wound healing. J. Biol. Chem. 274, 3228-3234.

Shiratori Y., Imazeki F., Moriyama M., Yano M., Arakawa Y., Yokosuka O., Kuroki T., Nishiguchi S., Sata M., Yamada G., Fujiyama S., Yoshida H. and Omata M. (2000). Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann. Intern. Med. 132, 517-524.

Sato M., Kakubari M., Kawamura M., Sugimoto J., Matsumoto K. and Ishii T. (2000). The decrease in total collagen fibers in the liver by hepatocyte growth factor after formation of cirrhosis induced by thioacetamide. Biochem. Pharmacol. 59, 681-690.

Sieg D.J., Hauck C.R. and Schlaepfer D.D. (1999). Required role of focal adhesion kinase (FAK) for integrin-stimulated cell migration. J. Cell Sci. 112, 2677-2691.

Stefanovic B., Hellerbrand C., Holcik M., Briendl M., Aliebhaber S. and Brenner D.A. (1997). Posttranscriptional regulation of collagen alpha1(I) mRNA in hepatic stellate cells. Mol. Cell. Biol. 17, 5201-5209.

Takahara T., Furui K., Yata Y., Jin B., Zhang L.P., Nambu S., Sato H., Seiki M. and Watanabe A. (1997). Dual expression of matrix metalloproteinase-2 and membrane-type 1-matrix metalloproteinase

in fibrotic human livers. Hepatology 26, 1521-1529.

Totsukawa G., Yamakita Y., Yamashiro S., Hartshorne D.J., Sasaki Y. and Matsumura F. (2000). Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. J. Cell Biol. 150, 797-806.

Ueki T., Kaneda Y., Tsutsui H., Nakanishi K., Sawa Y., Morishita R., Matsumoto K., Nakamura T., Takahashi H., Okamoto E. and Fujimoto J. (1999). Hepatocyte growth factor gene therapy of liver cirrhosis in rats. Nat. Med. 5, 226-230.

Ulloa L., Doody J. and Massague J. (1999). Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. Nature 397, 710-713.

Vasiliou V., Lee J., Pappa A. and Petersen D.R. (2000). Involvement of p65 in the regulation of NF-kappaB in rat hepatic stellate cells during cirrhosis. Biochem. Biophys. Res. Commun. 273, 546-550.

Wang Y.Q., Ikeda K., Ikebe T., Hirakawa K., Sowa M., Nakatani K., Kawada N. and Kaneda K. (2000) Inhibition of hepatic stellate cell proliferation and activation by the semisynthetic analogue of fumagillin TNP-470 in rats. Hepatology 32, 980-989.

Watanabe N., Kato T., Fujita A., Ishizaki T. and Narumiya S. (1999). Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. Nat. Cell Biol. 1, 136-143.

Watanabe T., Niioka M., Hozawa S., Kameyama K., Hayashi T., Arai M., Ishikawa A., Maruyama K. and Okazaki I. (2000). Gene expression of interstitial collagenase in both progressive and recovery phase of rat liver fibrosis induced by carbon tetrachloride. J. Hepatol. 33, 224-235.

Wells R.G. (2000). Fibrogenesis. V. TGF-beta signaling pathways. Am. J. Physiol. Gastrointest. Liver Physiol. 279, G845-G850.

Yanase M., Ikeda H., Matsui A., Maekawa H., Noiri E., Tomiya T., Arai M., Yano T., Shibata M., Ikebe M., Fujiwara K., Rojkind M. and Ogata I. (2000). Lysophosphatidic acid enhances collagen gel contraction by hepatic stellate cells: association with rho-kinase. Biochem. Biophys. Res. Commun. 277, 72-78.

Yee H.F. Jr. (2001). Ca^{2+} and rho signaling pathways: two paths to hepatic stellate cell contraction. Hepatology 33, 1007-1008.

Yee J.A., Yan L., Dominguez J.C., Allan E.H. and Martin T.J. (1993). Plasminogen-dependent activation of latent transforming growth factor beta (TGF beta) by growing cultures of osteoblast-like cells. J. Cell Physiol. 157, 528-534.

Yoshiji H., Kuriyama S., Miyamoto Y., Thorgeirsson U.P., Gomez D.E., Kawata M., Yoshii J., Ikenaka Y., Noguchi R., Tsujinoue H., Nakatani T., Thorgeirsson S.S. and Fukui H. (2000). Tissue inhibitor of metalloproteinases-1 promotes liver fibrosis development in a transgenic mouse model. Hepatology 32, 1248-1254.

Yu Q., Shao R., Qian H.S., George S.E. and Rockey D.C. (2000). Gene transfer of the neuronal NO synthase isoform to cirrhotic rat liver ameliorates portal hypertension. J. Clin. Invest. 105, 741-748.

Zhang W., Ou J., Inagaki Y., Greenwel P. and Ramirez F. (2000). Synergistic cooperation between Sp1 and Smad3/Smad4 mediates transforming growth factor beta1 stimulation of alpha 2(I)-collagen (COL1A2) transcription. J. Biol. Chem. 275, 39237-39245.

Zhang X., Chattopadhyay A., Ji Q.S., Owen J.D., Ruest P.J., Carpenter G. and Hanks S.K. (1999). Focal adhesion kinase promotes phospholipase C-gamma1 activity. Proc. Natl. Acad. Sci. USA 96, 9021-9026.

Zhou S., Zawel L., Lengauer C., Kinzler K.W. and Vogelstein B. (1998). Characterization of human FAST-1, a TGF beta and activin signal transducer. Mol. Cell. 2, 121-127.

Zhu J., Wu J., Frizell E., Liu S.L., Bashey R., Rubin R., Norton P. and Zern M.A. (1999). Rapamycin inhibits hepatic stellate cell proliferation in vitro and limits fibrogenesis in an in vivo model of liver fibrosis. Gastroenterology 17, 1198-1204.

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