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LIVER FIBROSIS: SIGNALS LEADING TO THE AMPLIFICATION OF THE FIBROGENIC HEPATIC STELLATE CELL

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1. ABSTRACT

Liver fibrosis represents a major medical problem with significant morbidity and mortality. Worldwide hepatitis viral infections represent the major cause liver fibrosis; however, within the United States chronic ethanol consumption is the leading cause of hepatic fibrosis. Other known stimuli for liver fibrosis include helminthic infection, iron or copper overload and biliary obstruction. Fibrosis can be classified as a wound healing response to a variety of chronic stimuli that is characterized by an excessive deposition of extracellular matrix proteins of which type I collagen predominates. This excess deposition of extracellular matrix proteins disrupts the normal architecture of the liver resulting in pathophysiological damage to the organ. If left untreated fibrosis can progress to liver cirrhosis ultimately leading to organ failure and death if left untreated. This review will discuss the molecular events leading to liver fibrosis. The discussion will include collagen gene regulation and proliferative signals that contribute to the amplification of the hepatic stellate cell, the primary fibrogenic cell type that resides in the liver.

2. INTRODUCTION

The hepatic stellate cell (HSC) (formerly called the Ito cell, fat storing cell, perisinusoidal cell, and lipocyte) is the primary cell-type in the liver responsible for excess collagen synthesis during hepatic fibrosis (1-10). Following a fibrotic stimulus, such as excess ethanol consumption, the HSC undergoes a complex transformation or activation process where the cell changes from a quiescent vitamin A storing cell to that of an activated myofibroblast-like cell (11-13). Cellular changes accompanying HSC activation include morphological changes to a myofibroblast-like cell that is associated with the appearance of the cytoskeletal protein smooth muscle α-actin, a loss in the cellular vitamin A stores, and an increase in the appearance of rough endoplasmic reticulum (2,5,8,10,14-21). Metabolically an increase in DNA synthesis and cellular proliferation occurs with HSC activation. Altered collagen synthesis, at both the mRNA and protein levels, is observed with a dramatic increase in type I collagen, and smaller but significant increases in type III and type IV collagens (3,5,8,10,14,15,17-23). Expression of HSP47, a collagen-binding stress protein believed to act as a collagen-specific molecular chaperone during collagen biosynthesis, is induced in the HSC during hepatic fibrosis (24). In addition, expression of all three isoforms of TGF-β, the most potent fibrogenic cytokine for HSCs, and its receptors are increased following HSC activation (25-27). Furthermore, the synthesis of platelet-derived growth factor-beta (PDGF-β), the most potent mitogen for HSCs is increased following HSC activation (6,24,25,28,29). In accordance, the PDGF-β receptor is also increased in activated HSCs (30,31).

Activation of HSCs results in two major events that promote the fibrogenic response of these cells. First, HSCs change their pattern of gene expression that results in an increase in synthesis and deposition of extracellular matrix proteins; hence these cells become directly fibrogenic. Secondly, the proliferation rate of HSCs increases following cellular activation. This effectively amplifies the number of fibrogenic cells present in the liver. Therefore, it is believed that effective treatment regimes aimed at reducing or inhibiting either the fibrogenic or

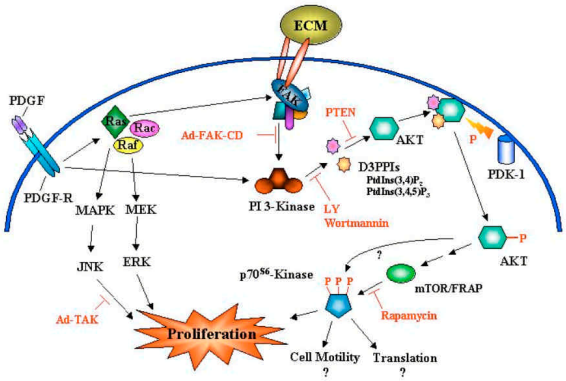

Figure 1. PDGF-Induced Proliferation Signaling in the Hepatic Stellate Cell. Following binding of PDGF to the PDGF cell surface receptor several signaling cascades are activated in the HSC. Activation of the MAPK-JNK pathway has been demonstrated as well as activation of the Ras-MEK-ERK pathway. In addition FAK has been shown to be involved in HSC proliferation which signals through the PI3-kinase-AKT-p70S6 kinase pathway. Cross-talk between the different signaling pathways also occurs.

proliferative responses of HSCs would reduce the deleterious effects of HSCs in the progression of fibrosis.

3. FIBROGENESIS

The molecular mechanisms underlying hepatic fibrosis are under extensive investigation. In the normal liver the turnover of the extracellular matrix (ECM) is maintained in a homeostatic equilibrium between ECM synthesis and degradation. During fibrosis an imbalance between ECM synthesis and degradation occurs. This imbalance results in a net increase in the synthesis and deposition of extracellular matrix proteins accompanied with an increase in tissue inhibitor of matrix metalloproteinase (TIMP) expression that inhibits collagenase activity.

The molecular mechanisms responsible for the increase in ECM components is best understood for type I collagen. Regulation of type I collagen during the fibrogenic response has shown to be a complex process. Type I collagen is the product of two genes, the α1(I) and α2(I) genes and although located on different chromosomes these genes are coordinately regulated in a tissue specific, developmental, and inducible manner. Increased type I collagen synthesis is due to both transcriptional and posttranscriptional mechanisms. An increase in the transcription rate of the α1(I) collagen gene is observed accompanied with an increase in Sp1 binding activity to the promoter (32-35). Sp1 is a potent transactivator of the α1(I) collagen gene (36). In addition, post-transcriptional regulation of the type I collagen takes place following HSC activation. An increase in the stability of the α1(I) collagen mRNA occurs that is mediated through protein interactions in the 3' untranslated region of the mRNA molecule. Increased translation of the α1(I) collagen message is noted following HSC activation that is mediated by protein binding to a conserved stem loop structure within the 5' region of the mRNA encompassing the translational start site (8,9,33,37-39). In addition to increased expression of collagen and other ECM components an increase in TIMP gene expression occurs as well as a decrease in matrix metalloproteinase gene expression.

There are several mediators that can influence the fibrogenic response of HSCs. These include retinoids, interleukin-1β, tumor necrosis factor, and acetaldehyde. However, transforming growth factor – beta (TGF-β) is the most potent fibrogenic cytokine known for the HSC. Exogenous administration of TGF-β induces fibrosis in the lung, kidney, and liver (40). During experimental models of hepatic fibrosis, including bile duct ligation, CCl_4 administration, and schistosomiasis infection, a prolonged increase in TGF-β expression is observed (41-44). In addition, patients with alcohol-induced and viral-induced cirrhosis also exhibit increased TGF-β mRNA levels that correlate with the extent of fibrosis (45-47). Studies using transgenic mice have provided strong evidence for a fibrogenic role of TGF-β in the development of liver fibrosis. A constitutively expressing form of active TGF-β resulted in increased levels of TGF-β with the development of hepatic fibrosis and increased type I collagen deposition (40,48,49).

4. PROLIFERATION

In addition to the molecular events leading to the excess synthesis and remodeling of the extracellular matrix, proliferation of activated HSCs amplifies the fibrogenesis. Quiescent HSCs reside in the liver in a non-proliferative state; however, once activated, either when cultured on plastic cellular or *in vivo* following a fibrogenic response, HSCs proliferate (50,51). Although several factors are mitogenic for HSCs the most potent mitogenic factors for the HSC is platelet-derived growth factor (PDGF) (52-58). PDGF is a heterodimeric protein composed of two polypeptide chains (A and B chains) that can combine into three different combinations, AA, BB, or AB. For the HSC, PDGF-BB is the most potent at stimulating cellular proliferation (32,53,59). The PDGF receptor is a transmembrane dimeric complex composed of αα, αβ, or ββ subunits (60). Binding of PDGF to its receptor results in receptor dimerization and activation by autophosphorylation of tyrosine residues (61). The phosphotyrosines on the activated receptor operate as high affinity binding sites for several molecules, mediated by either src homology domains (SH-2 domains) or phosphotyrosine binding domains that propagate the signals of receptor activation stimulating several signaling cascades (62,63) (see Figure 1).

4.1. MAPK signaling

Activation of Ras followed by the sequential activation of Raf, MEK, and ERK is one signaling cascade that is activated by PDGF stimulation of HSCs (54,64,65). ERK activation results in the phosphorylation of several transcription factors, including SAP and ELK-1. Activation of ERK is observed following PDGF stimulation in cultured HSCs and in HSCs isolated from animals treated with a single dose of CCl_4 (66). The

importance of this pathway in HSC proliferation was demonstrated when inhibition of ERK-1 ($p44^{MAPK}$) and ERK-2 ($p42^{MAPK}$) by pharmacological inhibitors markedly reduced growth factor stimulated gene transcription and cell proliferation (67). PDGF-induced ERK activation in human HSCs is followed by a transient up-regulation of c-fos expression and AP-1 and STAT1 binding activity to their cognate DNA binding sites (68,69). Furthermore, PDGF-induced generation of the lipid second messenger phosphatidic acid leads to a more sustained activation of ERK and subsequent proliferation (70). Blocking ERK activity, using the pharmacological inhibitor PD98059, inhibited HSC proliferation along with AP-1 and STAT1 DNA binding activities, thus supporting a role for ERK activation in HSC proliferation (66).

c-Jun and c-Jun nuclear kinase (JNK) are positive regulators of cell proliferation, including in HSCs (71-73). Blocking JNK activity in quiescent HSCs or in culture-activated HSCs using a dominant negative form of JNK (Ad-TAK) prevented increases in the cell population (74). Interestingly, inhibiting p38, another downstream MAP kinase target in either quiescent or activated HSCs, using the pharmacological inhibitor SB203580, actually increased cell proliferation, implying that activation of p38 inhibits HSC proliferation (74). An inhibitory role for p38 in cell proliferation has been shown in other cell types, perhaps by inhibiting cyclin D1 (75). Indeed, culture-induced proliferation of HSCs is associated with increased mRNA and protein levels of cyclins D1, D2, and E (76).

4.2. Modulation of PDGF signaling

PDGF may modulate its own proliferative effects. Both thrombin and PDGF generate high levels of prostaglandin E_2 (PGE_2) and cAMP that can counteract their proliferative effects. Increased cAMP levels and activation of PKA reduces PDGF stimulation of cellular proliferation (77). In HSCs, the phosphodiesterase inhibitor pentoxifylline increased cAMP levels and markedly decreased PDGF-induced ERK stimulation, mitogenesis, c-fos mRNA expression, and cytosolic Ca^{+2} (78). Similar results were obtained with isomethylbutyl xanthine, another phosphodiesterase inhibitor (79). Nitrovasodilators, which also increase PGE_2 levels showed a dose-dependent decrease in PDGF-induced elevation of intracellular Ca^{+2}, ERK activity, cell migration and DNA synthesis (80). Phosphorylation of Raf-1, an upstream activator of ERK, by protein-kinase A (PKA) may be responsible for the inhibitory effects of increased cAMP levels on PDGF stimulated cell growth (77,81). In addition, PKA also phosphorylates the transcription factor CREB at Ser133 that is associated with a reduction in HSC proliferation. In HSCs expressing a constitutively active form of PKA proliferation was inhibited; however, cells expressing CREB alone or those expressing a mutant form of CREB (CREB-Ala133) did not show anti-proliferative effects (79).

The anti-proliferative effects of PDGF may be mediated by ERK inhibition or by up-regulation of endothelin B receptors (ETB-R) amplifying the anti-proliferative effect of endothelin-1 in HSCs (82). Endothelin 1 inhibits proliferation of activated HSCs via ETB-R since endothelin induces an increase in prostaglandin synthesis and the associated increase in cAMP levels inhibits both ERK and JNK activity (83). A possible mechanism for cAMP-dependent HSC growth arrest is the inhibition of the transcription factor STAT1 (84). Another possible mechanism by which PDGF can inhibit its own proliferative response is the ability of cAMP to block activation of Raf-1, MEK, and MAPK by phosphorylation of Raf-1, thereby inhibiting its binding affinity to Ras (81). Phosphorylation of Raf-1 is mediated by PKA, which in turn is activated by cAMP (77).

4.3. PI3-kinase – Akt signaling

The phosphatidylinositol 3-kinase (PI3-kinase) – Akt pathway is also activated following PDGF stimulation of HSCs is (58,85). PI3-kinase is a heterodimeric protein that possesses lipid and protein kinase activity. It is composed of a regulatory 85 kDa subunit and a 110 kDa catalytic subunit. After PDGF stimulation, the 85 kDa subunit of PI3-kinase associates with the activated PDGF receptor through two SH-2 domains and becomes phosphorylated. Activation of PI3-kinase results in the generation of several phosphorylated inositol lipids (PtdIns), specifically PtdIns3P, PtdIns(3,4)P_2, PtdIns(3,5)P_2, and PtdIns(3,4,5)P_3, from phosphoinositols that are essential for intracellular signaling (86,87). The generated phosphorylated inositol lipids bind to the pleckstrin homology domain of Akt and induces Akt translocation to the plasma membrane. Once located at the plasma membrane, conformational changes occur that permit phosphoinositide-dependent kinase 1 (PDK-1) to phosphorylate Akt at Thr 308 and Ser 473 residues in the activation loop (87-90). Activated Akt is a key downstream survival factor by stimulating cell proliferation and inhibiting apoptosis (91-93). Akt can be activated not only by growth factors that trigger tyrosine kinase activity or activation of cytokine receptors, but also by other signals that can activate PI3-kinase including integrins and stimulators of G-protein-coupled receptors (87,94-97).

Activation of PI3-kinase is important for HSC proliferation and chemotaxis (85; our unpublished observations). A role for PI3-kinase in HSC proliferation is supported by *in vivo* studies in rats where CCl_4 treatment leads to autophosphorylation of the PDGF receptor and increased PI3-kinase activity. Furthermore, inhibition of PI3-kinase by wortmannin blocked mitogensis in response to PDGF supporting the involvement of this pathway in HSC proliferation (85). Similar results are obtained using a more specific PI3-kinase inhibitor LY294002 (65). Inhibition of PI3-kinase by wortmannin also reduces ERK activity and c-fos mRNA levels indicating cross-talk occurs between the PI3-kinase and MAP-kinase pathways following PDGF stimulation in HSCs (68). Over expression of a constitutively active form of Akt stimulates $p70^{S6K}$ and promotes cell proliferation and survival (93-95). Our laboratory has demonstrated that serum or PDGF activates Akt in HSCs by phosphorylating Ser 473. Inhibiting PI3-kinase using LY294002 blocks this activity. Akt phosphorylation correlated with an increase in HSC proliferation, α1(I) collagen mRNA levels, and type I

collagen protein levels, implicating its participation in HSC proliferation. Both inhibition of PI3-kinase with LY and inhibition of Akt by adenoviral mediated transduction of a dominant negative form of Akt markedly reduced HSC proliferation, α1(I) collagen mRNA, and protein levels. Transduction of HSCs with an adenovirus expressing a constitutively active form of Akt induced HSC proliferation in low serum conditions. Thus, it is concluded that Akt is positioned downstream of PI3-kinase (our unpublished observations).

4.4. FAK signaling

PDGF also activates focal adhesion kinase (FAK), a member of the focal adhesion complex (98). This complex interacts with extracellular matrix proteins through integrin interactions, providing a direct sensor to the integrity of the extracellular environment. PDGF treatment of HSCs leads to FAK phosphorylation, which is blocked by a dominant negative form of ras (99,100). We have shown that PDGF treatment activates and requires focal adhesion kinase (FAK) and PI3-kinase for HSC proliferation (unpublished observations). Blocking FAK activity using a dominant negative form of FAK (Ad-FAK-CD) inhibits HSC proliferation and PI3-kinase activity following PDGF treatment. We have also demonstrated that FAK is positioned upstream of PI3-kinase in the proliferative response of HSCs to PDGF. Additionally, blocking FAK inhibits cell migration (unpublished observations).

4.5. $p70^{S6}$ kinase signaling

Another downstream target in the PI3-kinase pathway via Akt is p70 S6 kinase ($p70^{S6}$ kinase). The $p70^{S6}$ kinase is a 70 kDa protein that is activated by mitogens and growth factors, several hormones including insulin, amino acids, and by intracellular calcium (101-105). A primary target of $p70^{S6}$ kinase is the ribosomal S6 protein of the 40S ribosomal subunit (106,107). $p70^{S6}$ kinase exists in two different isoforms, a 70 kDa cytoplasmic and an 85 kDa nuclear form. It is a key regulatory element of mRNA translation and protein synthesis. Furthermore, it is required for cell cycle progression, cell differentiation and cell growth. Both isoforms are in the mitogen-activated pathway, downstream of PI3-kinase. Activation of $p70^{S6}$ kinase occurs through a complex series of phosphorylation events on several serine or threonine residues via the Akt/PDK1 pathway (108,109). Phosphorylation of Thr 229 and Thr 389 are most critical for kinase function (110). Phosphorylation of these sites is inhibited by wortmannin, LY, rapamycin, and by amino acid deprivation (110-112). Rapamycin, a lipophylic bacterial macrolide, potently inhibits proliferation of yeast and several types of mammalian cells, including B and T lymphocytes (113,114). Rapamycin inhibits $p70^{S6}$ kinase activity by inhibiting mTOR/FRAP, an upstream kinase for $p70^{S6}$ kinase reducing phosphorylation of several regulatory sites on the $p70^{S6}$ kinase molecule and may activate serine, threonine protein phosphates (101,102). Rapamycin also blocked extracellular matrix deposition in CCl_4-induced liver fibrosis in rats. Moreover, rapamycin inhibits HSC proliferation due to PDGF stimulation in cell culture (109). Inhibition of $p70^{S6K}$ impairs the progression of cell cycle through G1 to S phase (115-117).

Rapamycin forms a complex with FKB12 that then inhibits the activity of the mammalian target of rapamycin (mTOR/FRAP), a pathway distinct from MAPK (106). In HSCs, rapamycin effectively blocked insulin-like growth factor-induced DNA synthesis (107). Yet, the mechanism by which rapamycin inhibits stellate cell proliferation has not been clarified. In HSCs, we were able to demonstrate that phosphorylation of $p70^{S6}$ kinase is induced by serum or PDGF and this activity is blocked by LY or rapamycin. Furthermore, LY and rapamycin blocked serum-induced HSC proliferation, α1(I) collagen mRNA and collagen protein levels (unpublished observations). These data indicate that $p70^{S6}$ kinase is positioned downstream of Akt in the PI3-kinase pathway and is strongly involved in HSC proliferation. We have shown that PDGF activates FAK and PI3-kinase in HSC proliferation (unpublished observations). Taken together, we have also shown FAK signals to PI3-kinase leading to Akt activation and subsequent $p70^{S6}$ kinase activation. Furthermore, rapamycin modulates the proliferative response in HSCs by inhibiting $p70^{S6}$ kinase (unpublished data). The likely mechanisms by which rapamycin blocks $p70^{S6}$ kinase activity is either by direct inhibition of $p70^{S6}$ kinase phosphorylation in the PI3-kinase pathway or by inhibition of the mTOR/FRAP induced phosphorylation (112,113).

4.6. Signaling complexity

In general, one signaling factor can be responsible for a wide range of possible biologic effects. This may be due to the complexity of intracellular signaling, because one signal does not strictly stimulate only a single cascade but more likely cross-talk with other pathways, therefore modulating a broad spectrum of signaling kinases. On the other hand a single signaling factor may have several biologic functions, indicating the diversity of cellular metabolism. However, pharmacologic blockade of one specific signaling step several hours after growth factor stimulation still can result in abrogation of the biologic response. This suggests that a stimulus needs a distinct amount of time until it results in the desired action. An early stage is probably the association of the growth factor with its receptor, followed by intermediate and late phase until completion of the biologic effect (114). In fact, it has been demonstrated that growth-factor-dependent mitogenesis requires two specific phases of signaling in the Ras/ERK pathway for progression in the cell cycle (101). This accounts also for PDGF-induced PI3-kinase activation, where only the late wave of activation is required for progression through G1 (102). Similar results were obtained in human HSCs, showing that PDGF-induced stimulation resulted in a biphasic stimulation of ERK activity, which was related to the stimulation of cell proliferation (103).

A proper intracellular milieu is essential for the correct execution of the biological function of all the described kinase pathways. Therefore, it is not surprising that pH and intracellular calcium concentration play a very important role in preserving homeostasis and kinase integrity in HSCs. For example, the mitogenic potential of the different isoforms of PDGF correlated with their

potential to increase intracellular Ca^{2+} in activated rat and human HSCs (104). Furthermore, the ability of PDGF to induce a proliferative response was associated with the capability to maintain an increased intracellular Ca^{+2} concentration due to Ca^{+2} entry (105,118). Accordingly, PDGF stimulation of HSCs in the absence of Ca^{2+} did not result in HSC proliferation (115). Regulation of the intracellular pH by modulating the activity of the Na^+/H^+ exchanger is another facet of PDGF (116,117). Pharmacologic inhibition of the Na^+/H^+ exchanger resulted in an inhibition of PDGF-induced mitogenesis, indicating that changes in the intracellular pH by this growth factor are essential for its proliferative activity (119). Furthermore, it has been proposed that PDGF-induced amplification of the Na^+/H^+ exchanger activity is linked to the activation of PI3-kinase and inhibition of the Na^+/H^+ exchanger lead to the disruption of downstream signaling events, such as PDGF-induced FAK phosphorylation (109). Indeed, we have demonstrated that FAK is involved in cell adhesion, cell migration and more importantly in HSC proliferation, because blocking FAK or PI3-kinase resulted in a marked reduction of these phenomena.

Finally, other mitogenic growth factors include angiogenic growth factors, such as basic fibroblast growth factor (b-FGF) and vascular endothelial growth factor (VEGF). In stellate cells b-FGF has a similar mitogenic and chemoattractant activity as PDGF (120). VEGF signaling in HSCs via its receptors includes activation of ERK, protein-kinase C and c-Akt. Activation of HSCs is associated with increased expression of VEGF and its receptors, which may again result in more cross-talk signals in the complex signal transduction pathways of hepatic stellate cells (121,122).

5. PERSPECTIVE

In summary, proliferation in hepatic stellate cells is regulated via different signaling cascades (see Figure 1). Recent research has led to better understanding of how HSCs change from a quiescent to an activated and proliferating cell type. However, further research needs to address the question of how the cell-cycle is targeted by these signaling pathways. It is anticipated that an understanding of the molecular events leading to fibrogenesis and HSC proliferation may lead to the development of effective therapies for hepatic fibrosis.

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7. REFERENCES

1. Maher J. J. & R. F. McGuier: Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis in vivo. *J Clin Invest* 86, 1641-1648 (1990)
2. Weiner F. R., M. A. Giambrone, M. J. Czaja, A. Shah, G. Annoni, S. Takahashi, M. Eghbali & M. A. Zern: Ito-cell gene expression and collagen regulation. *Hepatology* 11, 111-117 (1990)
3. Milani S, H. Herbst, D. Schuppan, E. G. Hahn & H. Stein: In situ hybridization for procollagen type I, III and IV mRNA in normal and fibrotic rat liver; evidence for predominant expression in nonparenchymal liver cells. *Hepatology* 10, 84-92 (1989)
4. Milani S, H. Herbst, D. Schuppan, K. Y. Kim, E. O. Riecken & H. Stein: Procollagen expression by nonparenchymal rat liver cells in experimental biliary fibrosis. *Gastroenterology* 98, 175-184 (1990)
5. Tsukamoto H., M. Matsuoka, W. Blaner & S. Cheng: Ito cell activation during progression of alcoholic liver fibrosis. *Cells of the Hepatic Sinusoid* 3, 453-456 (1991)
6. Nakatsukasa H., P. Nagy, R. P. Evart, H. Chu-Chieh, E. Marsden & S. S. Thorgiersson: Cellular distribution of transforming growth factor β1 and procollagen type I, III, and IV transcripts in carbon tetrachloride-induced rat liver fibrosis. *J Clin Invest* 85, 1833-1843 (1990)
7. Takahara T., T. Kohima, C. Miyabayashi, K. Inoue, H. Sasaki, Y. Muragai & A. Ooshima: Collagen production in fat-storing cells after carbon tetrachloride intoxication in the rat. Immunoelectron microscopic observation of type I, type III collagen and prolyl hydroxylase. *Lab Invest* 59, 509-521 (1988)
8. Shiratori Y., Y. Ichida, A. Geerts & E. Wisse: Modulation of collagen synthesis by fat-storing cells isolated from CCl-4 or vitamin A-treated rats. *Dig Dis Sci* 32, 1281-1289 (1987)
9. Friedman S. L.: Cellular sources of collagen and regulation of collagen production in liver. *Seminars Liver Dis* 10, 20-29 (1990)
10. Maher J. J., D. M. Bissell, S. L. Friedman & F. J. Roll. Collagen measured in primary cultures of normal rat hepatocytes derives from lipocytes within the monolayer. *J Clin Invest* 82, 450- 459 (1988)
11. Olaso E. & S. L. Friedman: Molecular regulation of hepatic fibrogenesis. *J Hepatol* 29, 836-847 (1998)
12. Friedman S. L.: Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 275, 2247-2250 (2000)
13. Eng F. J. & S. L. Friedman: Fibrogenesis. I. New insights into hepatic stellate cell activation: the simple becomes complex. *Am J Physiol Gastrointest Liver Physiol* 279, G7-G11 (2000)
14. Mak K. M., M. A. Leo MA & C. S. Lieber: Alcoholic liver injury in baboon: transformation of lipocytes to transitional cells. *Gastroenterology* 87, 188-200 (1984)
15. Ogawa K., J.-I. Suzuki, H. Mukai & M. Mori: Sequential changes of extracellular matrix and proliferation of Ito cells with enhanced expression of desmin and actin in focal hepatic injury. *Am J Pathol* 125, 611-619 (1986)
16. Ramadori G., T. Weit, S. Schwogler, H. P. Dienes, T. Knittel, H. Tieder & K.-H. Meyer zum Buschenfelde: Expression of the gene of the alpha-smooth muscle actin isoform in rat liver and in rat fat-storing (Ito) cells. *Virchows Archiv B* 59, 349-357 (1990)
17. Kawase T., Y. Shiratori & T. Sugimoto: Collagen production by rat liver fat-storing cells in primary culture. *Exp Cell Biol* 54, 183-192 (1986)
18. Geerts A., R. Vrijsen, J. Rauterberg, A. Burt, P. Schellinck & E. Wisse: In vitro differentiation of fat-storing cells parallels marked increase of collagen synthesis and secretion. *J Hepatology* 9, 59-68 (1989)

19. DeLeeuw A. M., S. P. McCarthy, A. Geerts & D. L. Knook: Purified rat liver fat storing cells divide in culture and contain collagen. *Hepatology* 4, 392-403 (1984)
20. Friedman S. L., D. C. Rockey, R. F. McGuire, J. J. Maher, J. K. Boyles & G. Yamasaki: Isolated hepatic lipocytes and kupffer cells from normal human liver: morphological and functional characteristics in primary culture. *Hepatology* 15, 234-243 (1992)
21. Knittel T., D. Schuppan, K.-H. Meyer zum Buschenfelde & G. Ramadori: Differential expression of collagen types I, III, IV by fat-storing (Ito) cells in vitro. *Gastroenterology* 102, 1721-1735 (1992)
22. Maher J. J., S. L. Friedman, F. J. Roll & D. M. Bissell: Immunolocalization of laminin in normal rat liver and biosynthesis of laminin by hepatic lipocytes in primary culture. *Gastroenterology* 94, 1053-1062 (1988)
23. Tsukamoto H., M. Matsuoka, W. Blaner & S. Cheng: Ito cell activation during progression of alcoholic liver fibrosis. In: Cells of the Hepatic Sinusoid, Vol 13. Eds: E. Wisse E., Knook D. L., McCuskey R. S., Kupffer Cell Foundation, the Netherlands.
24. Masuda H., M. Kukomoto, K. Hirayoshi & K. Magata: Coexpression of the collagen-binding stress protein HSP47 gene and the alpha 1(I) and alpha 1(III) collagen genes in carbon tetrachloride-induced rat liver fibrosis. *J Clin Invest* 94, 2481-2488 (1994)
25. Weiner F. R., A. Shah, L. Biempica, M. A. Zern & M. J. Czaja: The effects of hepatic fibrosis on Ito cell gene expression. *Matrix* 12, 36-43 (1992)
26. Gong W., S. Roth, K. Michel & A. M. Gressner: Isoforms and splice variant of transforming growth factor beta-binding protein in rat hepatic stellate cells. *Gastroenterology* 114, 352-363 (1998)
27. Friedman S. L., G. Yamasaki & L. Wong: Modulation of transforming growth factor beta receptors of rat lipocytes during the hepatic wound healing response. Enhanced binding and reduced gene expression accompany cellular activation in culture and in vivo. *J Biol Chem* 269, 10551-10558 (1994)
28. Marra F., G. G. Choudhury, M. Pinzani & H. E. Abboud: Regulation of platelet-derived growth factor secretion and gene expression in human liver fat-storing cells. *Gastroenterology* 107, 1110-1117 (1994)
29. Matsuoka M., N. Pham & H. Tsukamoto: Differential effects of interleukin-1-alpha, tumor necrosis factor-alpha, and transforming growth factor-beta-1 on cell proliferation and collagen formation by cultured fat-storing cell. *Liver* 9, 71-78 *(1989)*
30. Wong L., G. Yamasaki, R. J. Johnson & S. L. Friedman: Induction of platelet-derived growth factor receptor in rat hepatic lipocytes during cellular activation in vivo and in culture. *J Clin Invest* 94, 1563-1569 (1994)
31. Pinzani M., A. Gentilini, A. Caliqiuri, R. DeFranco, G. Pellegrini, S. Milani, F. Marra & P. Gentilini: Transforming growth factor-beta 1 regulates platelet-derived growth factor receptor beta subunit in human liver fat-storing cells. *Hepatology* 21, 232-239 (1995)
32. Pierce R. A., M. R. Bluag, R. S. Greco, J. W. Mackenzie, C. D. Boyd & S. B. Deak: Increased procollagen mRNA levels in carbon tetrachloride-induced liver fibrosis in rats. *J Biol Chem* 262, 1652-1658 (1987)
33. Panduro A., F. Shalaby, L. Biempica & D. A. Shafritz: Changes in albumin, alphafetoprotein and collagen gene transcription in CCl_4-induced hepatic fibrosis. *Hepatology* 8, 259-266 (1988)
34 Stefanovic B., C. Hellerbrand & D. A. Brenner: Post-transcriptional regulation of collagen alpha 1(I) mRNA in hepatic stellate cells. *Nucleic Acids Symp Ser* 33, 212-214 (1995)
35. Rippe R. A., G. Almounajed & D. A. Brenner: Sp1 binding activity increases in activated Ito cells. *Hepatology* 22, 241-251 (1995)
36. Nehls M. C., R. A. Rippe, L. Veloz & D. A. Brenner: Transcription factors NF-I and Sp1 interact with the murine collagen alpha 1(I) promoter. *Mol Cell Biol* 11, 4065-4073 (1991)
37. Stefanovic B., C. Hellerbrand & D. A. Brenner. Regulatory role of the conserved stem-loop structure at the 5' end of collagen alpha1(I) mRNA. *Mol Cell Biol* 19, 4334-4342 (1999)
38. Lindquist J. N., B. Stefanovic & D. A. Brenner: Regulation of collagen alpha1(I) expression in hepatic stellate cells. *J Gastroenterol* 35 Suppl 12, 80-83 (2000)
39. Lindquist J. N., W. F. Marzluff & B. Stefanovic: Fibrogenesis. III. Posttranscriptional regulation of type I collagen. *Am J Physiol (Gastrointest Liver Physiol)* 279, G471-G476 (2000)
40. Kanzler S., A. W. Lohse, A. Keil, J. Henninger, H. P. Dines, P. Schirmacher, S. Rose-John, K.-H. Meyer zum Buschenfelde & M. Blessing: TGB-β1 in liver fibrosis: an inducible transgenic mouse model to study liver fibrogenesis. *Am J Physiol* 39, G1059-1068 (1999)
41. De Bleser P. J., T. Kiki, V. Rogiers & A. Geerts: Transforming growth factor beta gene expression in normal and fibrotic rat liver. *J Hepatol* 26, 886-893 (1997)
42. Bissell D. M., S.-S. Wang, W. R. Jarnagin & F. J. Roll: Cell-specific expression of transforming growth factor beta in rat liver. Evidence for autocrine regulation of hepatocyte proliferation. *J Clin Invest* 96, 447-455 (1995)
43. Parola M., R. Muraca, I. Dianzani, G. Barrera, G. Leonarduzzi, P. Bendinelli, R. Piccoletti & G. Poli: Vitamin E dietary supplementation inhibits transforming growth factor beta 1 gene expression in the rat liver. *FEBS Lett* 308, 267-270 (1992)
44. Czaja M. J., F. R. Weiner, K. C. Flanders, M. A. Giambrone, R. Wind, L. Biempica & M. A. Zern: In vitro and in vivo association of transforming growth factor-β1 with hepatic fibrosis. *J Cell Biol* 108, 2477-2482 (1989)
45. Annoni G., F. Weiner & M. A. Zern: Increased transforming growth factor beta1 gene expression in human liver disease. *J Hepatol* 14, 259-264 (1992)
46. Bachem M. G., D. Meyer, R. Melchior, K. M. Sell & A. M. Gressner: Activation of rat liver perisinusoidal lipocytes by transforming growth factors derived from myofibroblast-like cells. A potential mechanism of self perpetuation in liver fibrogenesis. *J Clin Invest* 89, 19-27 (1992)
47. Castilla A., J. Prieto & N. Fausto: Transforming growth factors beta-1 and alpha in chronic liver disease – effects of interferon alfa therapy. *N Engl J Med* 324, 933-940 (1991)
48. Castilla A., J. Prieto & N. Fausto: Transforming growth factors beta-1 and alpha in chronic liver disease – effects of interferon alfa therapy. *N Engl J Med* 324, 933-940 (1991)
49. Clouthier D. E., S. A. Comerford & R. E. Hammer: Hepatic fibrosis, glomerulosclerosis, and a lipodystrophy-

like syndrome in PEPCK-TGF beta1 transgenic mice. *J Clin Invest* 100, 2697-2713 (1997)
50. Friedman S. L., F. J. Roll, J. Boyles, D. M. Arenson & D. M. Bissell: Maintenance of differentiated phenotype of cultured rat hepatic lipocytes by basement membrane matrix. *J Biol Chem* 264, 10756-10762 (1989)
51. Friedman S. L. & F. J. Roll: Isolation and culture of hepatic lipocytes, Kupffer cells, and sinusoidal endothelial cells by density gradient centrifugation with Stractan. *Anal Biochem* 161, 207-218 (1987)
52. Friedman S. L. & M. J. Arthur. Activation of cultured rat hepatic lipocytes by Kupffer cell conditioned medium. Direct enhancement of matrix synthesis and stimulation of cell proliferation via induction of platelet-derived growth factor receptors. *J Clin Invest* 84, 1780-1785 (1989)
53. Pinzani M., L. Gesualdo, G. M. Sabbah & H. E. Abboud: 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 (1989)
54. Pinzani P., F. J. Marra & V. Carloini: Signal transduction in hepatic stellate cells. *Liver* 18, 2-23 (1998)
55. Pinzani M., S. Milani, C. Grappone, F. J. Weber, P. Gentilini & H. E. Abboud: Expression of platelet-derived growth factor in a model of acute liver injury. *Hepatology* 19, 701-707 (1994)
56. Pinzani M., S. Milani, H. Herbest, R. DeFranco, C. Grappone, A. Gentilini, A. Caligiuri, G. Pellegrini, D. V. Nego, R. G. Romanelli & P. Gentilini: Expression of platelet derived growth factor and its receptor in normal human liver and during active hepatic fibrogenesis. *Am J Pathol* 148, 785-800 (1996)
57. Maher J. J.: Leukocytes as modulators of stellate cell activation. *Alcohol Clin Exp Res* 23, 917-921 (1999)
58. Fabio M., M. Pinzani, F. DeFranco, G. Laffi & P. Gentilini: Involvement of phosphatidylinositol 3-kinase in the activation of extracellular signal-regulated kinase by PDGF in hepatic stellate cells. *FEBS Lett* 336, 141-145 (1995)
59. Pinzani M., H. E. Abboud, L. Gesualdo & S. L. Abboud: Regulation of macrophage-colony stimulating factor in liver fat-storing cells by peptide growth factors. *Am J Physiol Cell Physiol* 262, C876-C881 (1992)
60. Seifert R. A., C. E. Hart, P. E. Phillips, J. W. Forstrom, R. Ross, M. J. Murray, D. F. Bowen-Pope: Two different subunits associate to create isoform-specific platelet-derived growth factor receptors. *J Biol Chem* 264, 8771-8778 (1989)
61. Claesson-Welsh L.: Platelet-derived growth factor receptor signals. *J Biol Chem* 269, 32023-32026 (1994)
62. Cohen G. B., R. Ren & D. Baltimore: Modular binding domains in signal transduction proteins. *Cell* 80, 237-248 (1995)
63. Pawson T.: Protein modules and signaling networks. *Nature* 373, 573-580 (1995)
64. Marshall C. J.: Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* 80, 179-185 (1995)
65. Gentilini A., F. Marra, P. Gentilini & M. Pinzani: Phosphatidlinostiol-3 kinase and extracellular signal-related kinase mediate the chemotactic and mitogenic effects of insulin-like growth factor-I in human hepatic stellate cells. *J Hepatol* 32, 227-234 (2000)
66. Marra F., M. C. Arrighi, M. Fazi, A. Caligiuri, M. Pinzani, R. G. Romanelli, E. Efsen, G. Laffi & P. Gentilini: 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 (1999)
67. Pages G., P. Lenormand, G. L'Allemain, J. C. Chambard, S. Meloche & J. Pouyssegur: Mitogen-activated protein kinases p42mapk and p44mapk are required for fibroblast proliferation. *Proc Natl Acad Sci USA* 90, 8319-8323 (1993)
68. Marra F., M. Pinzani, R. DeFranco, G. Laffi & P. Gentilini: Involvement of phosphatidylinositol 3-kinase in the activation of extracellular signal-regulated kinase by PDGF in hepatic stellate cells. *FEBS Lett* 376, 141-145 (1995)
69. Marra F., G. G. Choudhury, H. E. Abboud, H. L. Reeves, M. G. Thompson, C. L. Dack, A. D. Burt & C. P. Day: Interferon-gamma-mediated activation of STAT1alpha regulates growth factor-induced mitogenesis. *J Clin Invest* 98, 1218-1230 (1996)
70. Reeves H. L., M. G. Thompson, C. L. Dack, A. D. Burt & C. P. Day: The role of phosphatidic acid in platelet-derived growth factor-induced proliferation of rat hepatic stellate cells. *Hepatology* 31, 95-100 (2000)
71. Schreiber M., A. Kolbus, F. Piu, A. Szabowski, U. Mohle-Steinlein, J. Tian, M. Karin, P. Angel & E. F. Wagner: Control of cell cycle progression by c-Jun is p53 dependent. *Genes Dev* 13, 607-619 (1999)
72. Mitsui H., N. Takuwa, K. Kurokawa, J. H. Exton & Y. Takuwa: Dependence of activated G12-induced G1 to S phase cell cycle progression on both Ras/Mitogen-activated protein kinase and Ras/Rac1/Jun N-terminal kinase cascades in NIH3T3 fibroblasts. *J Biol Chem* 272, 4904-4910 (1997)
73. Bost F., R. McKay, N. Dean & D. Mercola: The JUN kinase/stress-activated protein kinase pathway is required for epidermal growth factor stimulation of growth of human A549 lung carcinoma cells. *J Biol Chem 272*, 33422-33429 (1997)
74. Schnabl B., C. A. Bradham, B. L. Bennett, A. M. Manning, B. Stefanovic & D. A. Brenner: TAK1/JNK and p38 have opposite effects on rat hepatic stellate cells. *Hepatology* 34, 953-963 (2001)
75. Lavoie J. N., G. L'Allemain, A. Brunet, R. Muller & J. Pouyssegur: Cyclin D1 expression is regulated positively by the p42/p44MAPK and negatively by the p38/HOGMAPK pathway. *J Biol Chem* 271, 20608-20616 (1996)
76. Kawada N., K. Ikeda, S. Seki & T. Kuroki: Expression of cyclins D1, D2 and E correlates with proliferation of rat stellate cells in culture. *J Hepatol* 30, 1057-1064 (1999)
77. Graves L. M., K. E. Bornfeldt, E. W. Raines, B. C. Potts, S. G. Macdonald, R. Ross & E. G. Krebs: Protein Kinase A Antagonizes Platelet-Derived Growth Factor-Induced Signaling by Mitogen-Activated Protein Kinase in Human Arterial Smooth Muscle Cells. *Proc Natl Acad Sci USA* 90, 10300-10304 (1993)
78. Pinzani M., F. Marra, A. Caligiuri, R. DeFranco, A. Gentilini, P. Failli & P. Gentilini: Inhibition by pentoxifylline of extracellular signal-regulated kinase

activation by platelet-derived growth factor in hepatic stellate cells. *Br J Pharmacol* 119, 1117-1124 (1996)
79. Houglum K., K. S. Lee & M. Chojkier: Proliferation of hepatic stellate cells is inhibited by phosphorylation of CREB on serine 133. *J Clin Invest* 99, 1322-1328 (1997)
80. Failli P., R. M. DeFranco, A. Caligiuri, A. Gentilini, R. G. Romanelli, F. Marra, G. Batignani, C. T. Guerra, G. Laffi, P. Gentilini & M. Pinzani: Nitrovasodilators inhibit platelet-derived growth factor-induced proliferation and migration of activated human hepatic stellate cells. *Gastroenterology* 119, 479-492 (2000)
81. Wu J., P. Dent, T. Jelinek, A. Wolfman, M. J. Weber & T. W. Sturgill: Inhibition of the EGF-activated MAP kinase signaling pathway by adenosine 3',5'-monophosphate. *Science* 262, 1065-1069 (1993)
82. Mallat A., C. Gallois, J. Tao, A. Habib, J. Maclouf, P. Mavier, A.-M. Préaux & S. Lotersztajn: 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 (1998)
83. Mallat A., A. M. Preaux, C. Serradeil-Le Gal, D. Raufaste, C. Gallois, D. A. Brenner, C. Bradham, J. Maclouf, V. Iourgenko, L. Fouassier, D. Dhumeaux, P. Mavier & S. Lotersztajn: Growth inhibitory properties of endothelin-1 in activated human hepatic stellate cells: a cyclic adenosine monophosphate-mediated pathway. Inhibition of both extracellular signal-regulated kinase and c-Jun kinase and upregulation of endothelin B receptors. *J Clin Invest* 98, 2771-2778 (1996)
84. Kawada N., M. Uoya, S. Seki, T. Kuroki & K. Kobayashi: Regulation by cAMP of STAT1 activation in hepatic stellate cells. *Biochem Biophys Res Commun* 233, 464-469 (1997)
85. Marra F., A. Gentilini, M. Pinzani, G. G. Chodhury, M. Parola, H. Herbest, M. U. Dianzani, G. Laffi, H. E. Abboud & P. Gentilini: Phosphatidylinositol 3-kinase is required for platelet derived growth factor's actions on hepatic stellate cells. *Gastroenterology* 112, 1297-1306 (1997)
86. Parker P. J. & M. D. Waterfield: Phosphatidylinositol 3-kinase: a novel effector. *Cell Growth Differ* 3, 747-752 (1992)
87. Data S. R., A. Brunet & M. Greenberg: Cellular survival: a play in three Akts. *Genes & Development* 13, 905-2927 (1999)
88. Vanhaesebroeck B. & D. A. Aless: The PI3K connection: more than just a road to PKB. *Biochem J* 346, 561-576 (2000)
89. Tocker A.: Protein kinase as mediators of phosphoionositide 3-kinase signaling. *Mol Pharmacol* 57, 652-658 (2000)
90. Chan T. O., S. Rittenhouse & P. Tschils: Akt/PKB and other D3 phosphoinositide-regulated kinases: kinase activation by phosphoinositide dependent phosphorylation. *Annu Rev Biochem* 68, 661-664 *(1999)*
91. Kim A. L., G. Khursigara, X. Sun, T. F. Franke & M. V. Chao: Akt phosphorylates and negatively regulates apoptosis signal-regulating kinase 1. *Mol Cell Biol* 21, 893-901 (2001)
92. Bulik G., A. Klippel & M. J. Weber. Antiapoptotic signaling by the insulin-like growth factor I receptor, phosphatidylinositol 3-kinase, and Akt. *Mol Cell Biol* 17, 1595-1606 (1997)
93. Coffer P. J., J. Jin & J. R. Woodgett: Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. *Biochem J* 335, 1-13 (1998)
94. Khwaja A., P. Rodriguez-Viciana, S. Wennstrom, P. H. Warne, & J. Downward: Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. *EMBO J* 16, 2783-2793 (1997)
95. Shaw M., P. Cohen & D. R. Alessi: The activation of protein kinase B by H2O2 or heat shock is mediated by phosphatidylinositol 3-kinase and not by mitogenic-activated protein kinase. *Biochem J* 336, 241-246 (1998)
96. King W. G., M. D. Mattaliano, T. O. Chan, P. N. Tsichlis & J. S. Brugge: Phosphatidylinositol 3-kinase is required for integrin-stimulated AKT and Raf-1/mitogen-activated protein kinase pathway activation. *Mol Cell Biol* 17, 4406-4418 (1997)
97. Muraga C., S. Fukuhara & J. S. Gutkind: A novel role for phosphatidylinositol 3-kinase β in signaling from G protein-coupled receptors to Akt. *J Biol Chem* 275, 12069-12073 (2000)
98. Carloni V., R. G. Romanelli, M. Pinzani, G. Laffi & P. Gentilini: Focal adhesion kinase and phospholipase C? involvement in adhesion and migration of human hepatic stellate cells. *Gastroenterology* 112, 522-531 (1997)
99. Carloni V., R. G. Romanelli, M. Pinzani, G. Laffi & P. Gentilini: Focal adhesion kinase and phospholipase C ? involvement in cell adhesion and migration of human hepatic stellate cells. *Gastroenterology* 112, 522-531 (1997)
100. Carloni V., M. Pinzani, S. Giusti, R. G. Romanelli, M. Parola, G. Bellomo, P. Failli, A. D. Hamilton, S. M. Sebti, G. Laffi & P. Gentilini: Tyrosine phosphorylation of focal adhesion kinase mediates the integrin signaling requirement for growth factor activation of MAP kinase. *J Cell Biol* 147, 611-618 (1999)
101. Jones S. M. & A. Kazlauskas: Growth-factor-dependent mitogenesis requires two distinct phases of signaling. *Nat Cell Biol* 3, 165-172 (2001)
102. Jones S. M., R. Klinghoffer, G. D. Prestwich, A. Toker & A. Kazlauskas: PDGF induces an early and a late wave of PI 3-kinase activity, and only the late wave is required for progression through G1. *Curr Biol* 9, 512-521 (1999)
103. Bonacchi A., P. Romagnani, R. G. Romanelli, E. Efsen, F. Annunziato, L. Lasagni, M. Francalanci, M. Serio, G. Laffi, M. Pinzani, P. Gentilini & F. Marra: Signal transduction by the chemokine receptor CXCR3: activation of Ras/ERK, Src, and phosphatidylinositol 3-kinase/Akt controls cell migration and proliferation in human vascular pericytes. *J Biol Chem* 276, 9945-9954 (2001)
104. Pinzani M., T. C. Knauss, G. F. Pierce, P. Hsieh, W. Kenney, G. R. Dubyak & H. E. Abboud: Mitogenic signals for platelet-derived growth factor isoforms in liver fat-storing cells. *Am J Physiol* 260, C485-C491 (1991)
105. Kondo T., F. Konishi, H. Inui & T. Inagami: Differing signal transductions elicited by three isoforms of platelet-derived growth factor in vascular smooth muscle cells. *J Biol Chem* 268, 4458-4464 (1993)
106. Brown E. J., M. W. Albers, T. B. Shin, K. Ichikawa, C. T. Keith, W. S. Lane & S. L. Schreiber: A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature* 369, 756-758 (1994)

107. Skrtic S., K. Wallenius, A. M. Gressner & J. O. Jansson: Insulin-like growth factor signaling pathways in rat hepatic stellate cells: importance for deoxyribonucleic acid synthesis and hepatocyte growth factor production. *Endocrinology* 140, 5729-5735 (1999)
108. Dumont F. J., M. J. Staruch, S. L. Koprak, M. R. Melino & N. H. Sigal: Distinct mechanisms of suppression of murine T cell activation by the related macrolides FK-506 and rapamycin. *J Immunol* 144, 251-258 (1990)
109. Zhu J., J. Wu, E. Frizell, S. L. Liu, R. Bashey, R. Rubin, P. Norton & M. A. Zern: Rapamycin inhibits hepatic stellate cell proliferation in vitro and limits fibrogenesis in an in vivo model of liver fibrosis. *Gastroenterology* 117, 1198-1204 (1999)
110. Pullen N. & G. Thomas: The modular phosphorylation and activation of p70s6k. *FEBS Lett* 410, 78-82 (1997)
111. Polakiewicz R. D., S. M. Schieferl, A. C. Gingras, N. Sonenberg & M. J. Comb: mu-Opioid receptor activates signaling pathways implicated in cell survival and translational control. *J Biol Chem* 273, 23534-23541 (1998)
112. Jefferies H. B., S. Fumagalli, P. B. Dennis, C. Reinhard, R. B. Pearson & G. Thomas: Rapamycin suppresses 5′TOP mRNA translation through inhibition of p70s6k. *EMBO J* 16, 3693-3704 (1997)
113. Gingras A. C., B. Raught & N. Sonenberg: Regulation of translation initiation by FRAP/mTOR. *Genes Dev* 15, 807-826 (2001)
114. Pinzani M. & F. Marra: Cytokine receptors and signaling in hepatic stellate cells. *Semin Liver Dis* 21, 397-416 (2001)
115. Failli P., C. Ruocco, R. De Franco, A. Caligiuri, A. Gentilini, A. Giotti, P. Gentilini & M. Pinzani: The mitogenic effect of platelet-derived growth factor in human hepatic stellate cells requires calcium influx. *Am J Physiol* 269, C1133-C1139 (1995)
116. Di Sario A., G. S. Baroni, E. Bendia, L. D'Ambrosio, F. Ridolfi, J. R. Marileo, A. M. Jezequel & A. Benedetti: Characterization of ion transport mechanisms regulating intracellular pH in hepatic stellate cells. *Am J Physiol* 273, G39-G48 (1997)
117. Di Sario A., G. Svegliati Baroni, E. Bendia, F. Ridolfi, S. Saccomanno, L. Ugili, L. Trozzi, M. Marzioni, A. M. Jezequel, G. Macarri & A. Benedetti: Intracellular pH regulation and Na+/H+ exchange activity in human hepatic stellate cells: effect of platelet-derived growth factor, insulin-like growth factor 1 and insulin. *J Hepatol* 34, 378-385 (2001)
118. Wang Z., M. Estacion & L. J. Mordan: Ca2+ influx via T-type channels modulates PDGF-induced replication of mouse fibroblasts. *Am J Physiol* 265, C1239-C1246 (1993)
119. Benedetti A., A. Di Sario, A. Casini, F. Ridolfi, E. Bendia, P. Pigini, C. Tonnini, L. D'Ambrosio, G. Feliciangeli, G. Macarri & G. Svegliati-Baroni: 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 (2001)
120. Fibbi G., M. Pucci, C. Grappone, G. Pellegrini, R. Salzano, A. Casini, S. Milani & M. Del Rosso: Functions of the fibrinolytic system in human Ito cells and its control by basic fibroblast and platelet-derived growth factor.
121. Ishikawa K., S. Mochida, S. Mashiba, M. Inao, A. Matsui, H. Ikeda, A. Ohno, M. Shibuya & K. Fujiwara: Expression of vascular endothelial growth factor in nonparenchymal as well as parenchymal cells in rat liver after necrosis. *Biochem Biophys Res Commun* 254, 587-593 (1999)
122. Ankoma-Sey V., M. Matli, K. B. Chang, A. Lalazar, D. B. Donner, L. Wong, R. S. Warren & S. L. Friedman: Coordinated induction of VEGF receptors in mesenchymal cell types during rat hepatic wound healing. *Oncogene* 17, 115-121 (1998)

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