Regulation of TGF-β1-induced pro-apoptotic signaling by growth factor receptors and extracellular matrix receptor integrins in the liver

Iwata Ozaki1,2*, Hiroshi Hamajima2, Sachiko Matsushashi2 and Toshihiko Mizuta2

1 Saga Medical School, Health Administration Center, Saga, Japan
2 Division of Hepatology, Saga Medical School, Department of Internal Medicine, Saga University, Saga, Japan

INTRODUCTION

Hepatocellular carcinoma (HCC) often arises from chronically diseased livers. Persistent liver inflammation causes the accumulation of excessive extracellular matrix (ECM) proteins and impairs the liver function, finally leading to the development of HCC. A pleiotropic cytokine, transforming growth factor (TGF)-β1, plays critical roles throughout the process of fibrogenesis and hepatocarcinogenesis. In the liver, TGF-β1 inhibits the proliferation of hepatocytes and stimulates the production of ECM from hepatic stellate cells (HSCs) to maintain tissue homeostasis. During disease progression, both growth factors/cytokines and the ECM alter the TGF-β1 signals by modifying the phosphorylation of Smad proteins at their C-terminal and linker regions. TGF-β1 stimulates the expression of integrins, cellular receptors for ECM, along with an increase in ECM accumulation. The activation of integrins by the ECM modulates the response to TGF-β1 in hepatic cells, resulting in their resistance to TGF-β1-induced growth suppression in hepatocytes and the sustained production of ECM proteins in activated HSCs/myofibroblasts. Both growth factor receptors and integrins modify the expression and/or functions of the downstream effectors of TGF-β1, resulting in the escape of hepatocytes from TGF-β1-induced apoptosis. Recent studies have revealed that the alterations of Smad phosphorylation that occur as the results of the crosstalk between TGF-β1, growth factors and integrins modify the expression and/or functions of the downstream effectors of TGF-β1 in hepatic cells, resulting in their resistance to TGF-β1 signals from tumor suppression to promotion. Therefore, the modulation of Smad phosphorylation could be an attractive target for the prevention and/or treatment of HCC.

Keywords: TGF-β1, Smad, liver fibrosis, integrins, hepatocellular carcinoma

Abbreviations: CDK, cyclin-dependent kinase; ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial to mesenchymal transition; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HSC, hepatic stellate cells; IFN, interferon; IGF, insulin-like growth factor; IL, interleukin; ILK, integrin-linked kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MFB, myofibroblast; PDCD4, programmed cell death 4; PDGF, platelet-derived growth factor; PI3K, phosphoinositide-3-kinase; PKC, protein kinase C; pSmadC, C-terminally phosphorylated Smad; pSmadL, linker-phosphorylated Smad; TGF-β1; transforming growth factor-β; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.
pathways, such as extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK) in several cell types. TGF-β1 can also stimulate the phosphoinositide-3 kinase (PI3K)-Akt pathway and Rho-like GTPases (Figure 1).

In the liver TGF-β1 is a potent growth inhibitor of normal hepatocytes and HCC cells, and induces apoptosis and cellular senescence in these cells (Lin and Chou, 1992; Oberhammer et al., 1992; Siegel and Massague, 2003; Senturk et al., 2010). However, TGF-β1 is highly expressed in many malignant tumors, including HCC, and tumor cells are frequently thought to have lost their sensitivity to TGF-β1 (Ito et al., 1991; Bedossa et al., 1995; Tsai et al., 1997; Caja et al., 2007). During the disease progression, numerous growth factors/inflammatory cytokines such as TGF-β1, hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), interleukins (ILs), and interferons (IFNs) are released from various types of hepatic cells and participate in apoptosis, inflammation, cell proliferation, tissue fibrosis/remodeling, and HCC development/progression (Inagaki and Okazaki, 2007; Friedman, 2008). While TGF-β1 inhibits hepatocyte proliferation in the early stage of liver injury, it stimulates the proliferation of fibroblasts and induces the expression of ECM genes, including fibrillar collagens, to repair the injured tissue. When injurious stimulation is repeated, as occurs during persistent hepatitis virus infection, the production of ECM continues, and fibrous materials accumulate, thus leading to the formation of cirrhosis that predisposes the liver to HCC (El-Serag and Rudolph, 2007; Giannelli et al., 2011; Matsuzaki, 2011; Figure 2). Along with the progression of fibrogenesis, an increased expression of integrin, a cell surface receptor for the ECM, is observed (Volpes et al., 1991; Couvelard et al., 1993; Levine et al., 2000). Integrins interact with growth factor receptors, including TGF-β1 as well as with ECM proteins, and can modulate the signal transduction pathways (Hayashida, 2010; Margadant and Sonnenberg, 2010). Recent data indicate that during the progression of chronic hepatitis, from cirrhosis to HCC, changes in the TGF-β1-induced Smad phosphorylation pattern occur, and the alternatively phosphorylated Smad proteins (pSmadL) exert distinct transcriptional activities (Matsuzaki et al., 2007; Matsuzaki, 2011).

In this manuscript we review the effects of growth factor-mediated signals and integrin-mediated signals on the tumor suppressive signals induced by TGF-β1 in liver epithelial cells and discuss the mechanisms regulating the phosphorylation of the Smad protein as potential therapeutic targets.

**LIVER FIBROSIS, HCC, AND INTEGRINS**

Among the various growth factors and proinflammatory cytokines involved in the long-lasting process of liver fibrogenesis and hepatocarcinogenesis, TGF-β1 plays critical roles throughout the progression of the disease (Inagaki and Okazaki, 2007; Friedman, 2008; Giannelli et al., 2011; Matsuzaki, 2011). Once the liver injury is initiated and continued, hepatic stellate cells (HSCs), the main source of ECM production in the liver, are activated by TGF-β1 and proinflammatory cytokines. Sustained expression of ECM genes by TGF-β1 in the activated HSCs and myofibroblasts leads to the accumulation of collagenous ECM proteins (Furukawa et al., 2003; Inagaki and Okazaki, 2007; Matsuzaki, 2011).

TGF-β1 is known to increase the expression of integrins, major cellular receptors for ECM proteins, as well as its ligands, the ECM proteins (Hayashida, 2010; Margadant and Sonnenberg, 2010). With the progression of liver fibrosis, the expression of integrins is also increased (Couvelard et al., 1993; Volpes et al., 1993; Levine et al., 2000). The integrins are large family of heterodimeric cell surface receptors consisting of 18 α- and 8 β-transmembrane subunits. Integrins can directly bind to ECM components and transmit the signals from the ECM to the cell interior and vice versa (Hynes, 2002). The short cytoplasmic tails of integrins are associated with adaptor proteins including Src, focal adhesion kinase (FAK), integrin-linked kinase (ILK), kindlin1, paxilin, talin, vinculin, and PINCH. These integrin-associated proteins not only transmit signals from the extracellular environment, but also increase integrin activation. Integrin also interacts with cytoskeletal proteins and can regulate the cellular shape and motility upon activation (Figure 3). Among the various integrin-associated proteins, kinases such as Src, FAK, or ILK activate the cellular signal transduction pathways including MAP kinase pathways (ERK, p38, JNK), PI3K/Akt pathways, and protein kinase C (PKC) cascades (Hynes, 2002; Hehlgens et al., 2007; Bottcher et al., 2009; Millard et al., 2011). Integrins have also been shown to interact with growth factor receptors including IGF-IR, PDGFR-β, c-Met, and EGFR (Alam et al., 2007; Desgroisselier and Cheresh, 2016; Ivaska and Heino, 2010). Similarly, integrins can modulate the TGF-β1/Smad pathways directly or indirectly via different mechanisms, while TGF-β1 stimulates the expression of integrins (Margadant and Sonnenberg, 2010).
Ozaki et al. Modulation of TGF-β1 signal by growth factor and integrins

Figure 2 | Transforming growth factor-β1, fibrosis, and HCC. When hepatocytes are injured by agents such as hepatitis viruses, various growth factors, and proinflammatory cytokines, as well as TGF-β1 are released from non-parenchymal liver cells. In the earlier stages, growth factors stimulate the proliferation of hepatocytes to restore the liver function, while TGF-β1 induces the apoptosis of injured hepatocytes to maintain tissue homeostasis. If the injurious stimuli are persistent, ECM protein production from the activated HSCs/myofibroblasts is continuously stimulated. Both persistent inflammation and the accumulated ECM proteins contribute to the alteration of signal transduction pathways in regenerated hepatocytes, along with the accumulation of genetic mutations, which leads to the preneoplastic changes of hepatocytes and the eventual development of HCC.

Figure 3 | Integrins, adaptor proteins, and signaling pathways. When ECMs bind to integrin receptors and form focal adhesions, the cytoplasmic tails of β-integrins are associated with a number of adaptor proteins. Talin forms a contact with both the β-integrin tail and the actin cytoskeleton. ILK associates with actin through its partners such as parvin, PINCH and kindling. FAK is responsible for the binding of integrin-associated proteins like paxillin and talin. These integrin-associated proteins involve non-receptor type kinases, such as Src, FAK, or ILK, which activate cellular transduction pathways, including the MAP kinase pathways, PI3K/Akt pathways and PKCs. Integrins are also associated with cytoskeletal proteins through the adaptor proteins, and can regulate cellular shape and motility.

Based on this information, it has been speculated that an increased expression of integrins on the hepatocytes in a fibrotic liver could also modulate the intracellular signaling pathways and contribute to the development of HCC. The expression of several integrin subunits, including the α1, α2, α3, α5, α6, and β1 chains, is also upregulated in HCC cells, and their expression patterns are distinct from those of non-cancerous liver tissue (Volpes et al., 1993; Begum et al., 1995; Ozaki et al., 1998; Lai et al., 2011). Furthermore integrin α6 chain expression is correlated with a poor prognosis for HCC patients (Ke et al., 2011). Integrins α1β1, α2β1, α3β1, and α6β1 play key roles in the invasion of HCC cells (Carloni et al., 2001; Giannelli et al., 2001; Torimura et al., 2001; Yang et al., 2006) and integrins α3β1 and α6β4 promote the growth of HCC cells upon activation by their ligand, laminin-5 (Bergamini et al., 2007). These data strongly suggest the important roles of the cell-ECM interactions through integrin receptors in the progression of HCC.

Mechanisms of Resistance to TGF-β1-Induced Apoptosis in Liver Epithelial Cells

Transforming growth factor-β1 has a dual role in the development and progression of HCC. TGF-β1 is a potent growth inhibitor of normal hepatocytes and HCC cells and induces apoptosis in these cells, and as a result, it is regarded as a tumor-suppressive cytokine. However, TGF-β1 is able to promote the growth, invasion, and metastasis of HCC cells through the epithelial-mesenchymal transition (EMT; Bierie and Moses, 2006; Massague, 2008; Matsuzaki, 2011). The ability of TGF-β1 to inhibit or stimulate cancer progression is partly dependent on the tumor microenvironment, including the presence of growth factors/inflammatory cytokines and/or ECM proteins. During the progression of liver fibrogenesis with chronic inflammation, the hepatocyte responsiveness to...
TGF-β1 changes, and cells become resistant to TGF-β1-induced apoptosis, thus suggesting the existence of preneoplastic changes of hepatocytes in cirrhotic livers (Ito et al., 1991).

**GROWTH FACTORS AND TGF-β1-INDUCED APOPTOSIS**

The presence of growth factors such as HGF and EGF that stimulate hepatocyte proliferation has been shown to affect the TGF-β1-induced growth-suppressive signal in hepatocytes (Fabregat et al., 1996; Murillo et al., 2005). Similar effects of growth factors on TGF-β1 signals were observed in TGF-β1-sensitive HCC cells (Mori et al., 2004; Caja et al., 2007). When TGF-β1 induces apoptosis in hepatic cells, several downstream molecules are involved in the induction of apoptosis. For example, the activation of p38 by GADD45β (Yoo et al., 2003) and JNK activation by the Daxx protein (Perlman et al., 2001) were reported to be involved in TGF-β1-induced apoptosis in hepatocytes. In the human HCC cell line, Hep3B, DAP kinase was reported to be induced by TGF-β1 in a Smad-dependent manner, and to promote the apoptosis by releasing cytochrome c from the mitochondria (Jang et al., 2002). We recently reported that programmed cell death 4 (PDCD4) is expressed in certain HCC cell lines, such as HepG2 (Tajima et al., 1991; Shiota et al., 1992). When β1-integrin is overexpressed in HepG2 cells, HGF promotes the growth of the cells overexpressing β1-integrin, while the growth of wild-type and mock-transfected cells are suppressed by HGF (Zhang et al., 2003). β1-integrin suppressed the induction of p27 by HGF, a CDK inhibitor that inhibits cell cycle progression, and also promoted the proteasomal degradation of p27 by increasing the expression of its E3-ubiquitin ligase, Skp2. These findings indicate that β1-integrin-mediated signals from the ECM and growth factor receptor-mediated signals are cooperatively controlled and regulate the cellular functions and behavior.

**INTEGRINS AND TGF-β1-INDUCED APOPTOSIS**

The ECM has been used as a substrate for hepatocyte culture, and the presence of cell-matrix interactions in vitro was found to contribute to the survival and the maintenance of the differentiated functions of primary hepatocytes (Gómez-Lechón et al., 1998; Hansen and Albrecht, 1999; Cukierman et al., 2001; Godoy et al., 2009). Godoy et al. (2009) reported that the presence of ECM collagen inhibited the apoptosis of primary cultured hepatocytes induced by TGF-β1 in a Src/FAK-mediated Akt and p38-dependent manner. During the progression of liver fibrosis, TGF-β1 stimulates the expression of both ECMs and integrins (Inagaki and Okazaki, 2007; Hayashida, 2010). The activation of integrins by the ECM in the liver reciprocally alters the response of cells to TGF-β1, inducing resistance to TGF-β1-induced growth suppression in hepatocytes, and the sustained production of ECM proteins in activated HSCs and myofibroblasts. This interaction might further enhance the survival of hepatocytes and ECM production from HSCs by establishing a feed-forward loop between apoptosis resistance and fibrosis, and may therefore contribute to the progression from liver fibrosis to cirrhosis, and the development of preneoplastic changes in hepatocytes (Figure 4).

The resistance to apoptosis induced by ECM/integrins is also observed in cancer cells. The presence of the ECM and activation of integrin-mediated signals has been shown to inhibit cytotoxic agent-induced apoptosis in a MAP kinase-dependent manner (Sethi et al., 1999; Zhang et al., 2002). We have previously shown that human HCC cells overexpressing β1-integrin become resistant to TGF-β1-induced apoptosis compared to mock-transfected cells in a MAP kinase-dependent manner (Zhang et al., 2004). TGF-β1 is known to transiently activate the MAP kinase pathway in a Smad-independent manner (Derynck and Zhang, 2003; Mostakas and Heldin, 2005; Giannelli et al., 2011). An overexpression of β1-integrin in HCC cells was shown to induce the activation of the MAP kinase pathway, and TGF-β1-induced the further sustained activation of ERK, p38, and JNK. The inhibition of these pathways reversed the TGF-β1-induced apoptosis, suggesting that the MAP kinase pathway has critical roles in the escape of cells from TGF-β1-induced apoptosis (Zhang et al., 2004).

Furthermore, recent reports have suggested that the physical properties of the ECM, including the matrix stiffness, modulate the intracellular signaling and cellular behavior (Wells, 2008; Tilghman et al., 2010; Zhao et al., 2010; Schrader et al., 2011). Clinical observations indicated that the liver stiffness, as measured by transient elastography, was closely associated with the degree of liver fibrosis and cirrhosis, and could predict the development of HCC in patients with viral hepatitis (Masuzaki et al., 2009; Jung et al., 2011). Primary cultured hepatocytes with a stiff collagen
Alterations of the Smad phosphorylation status during the progression of chronic liver disease

When TGF-β1 exerts growth-inhibitory effects on epithelial cells, the TβRI-mediated phosphorylation of Smad TFs (Smad2/3) occurs at their C-terminal region (SSXS motif). Recent studies have shown that the linker region of Smad proteins could be phosphorylated by various kinases, including MAP kinases (ERK, p38, JNK), CDK, and ROCK (Kretzschmar et al., 1999; Matsuura et al., 2004; Mori et al., 2004; Kamaraju and Roberts, 2005; Wrighton et al., 2009; Burch et al., 2011) to modulate the transcriptional activity of the Smad complex, resulting in the escape of cells from apoptosis and the induction of EMT that contributed to tumor development and progression.

During the chronic inflammation of the liver, altered phosphorylation patterns of the Smad2 and Smad3 proteins are observed. Along with the progression of chronic liver disease, the site of phosphorylation in the Smad proteins induced by TGF-β1 is shifted from the C-terminal region (pSmadC) to the linker region (pSmadL) in activated HSCs and hepatocytes (Yoshida et al., 2005; Matsuzaki et al., 2007; Murata et al., 2009; Matsuzaki, 2011). Phosphorylation of the linker region of Smad2/3 in HCCs is caused by the activation of JNK through PDGF, leading to the sustained activation of HSCs, differentiation to myofibroblast-like cells and continued production of collagen-rich ECM. In hepatocytes in the earlier stages of chronic liver disease, TGF-β1 induces the C-terminal phosphorylation of Smad (pSmadC). However, in the later stage of chronic liver disease when fibrosis in the liver has advanced, the linker-pSmadL in hepatocytes become prominent, and TGF-β1-induced growth inhibition is attenuated. The loss of TGF-β1-induced growth inhibition might be a marker for the pre-neoplastic changes of hepatocytes, and the dominance of pSmadL could be a useful indicator of the development of HCC (Matsuzaki et al., 2007; Murata et al., 2009; Matsuzaki, 2011).

In HCC cells, alterations in the Smad phosphorylation patterns modify the cellular responses to TGF-β1. Blockade of the C-terminal phosphorylation of Smad2 in HCC cells by transfecting mutant plasmids in which the C-terminal serine residues are substituted with alanine inhibited TGF-β1-induced pro-apoptotic Smad-mediated transcriptional activities (Sugano et al., 2003). Both growth factors and integrins regulate the phosphorylation pattern of Smads and modulate the cellular behavior. The presence of ECM proteins or overexpression of β1-integrin both induced the phosphorylation of the linker region of Smad2 and Smad3 and blocked the TGF-β1-induced C-terminal phosphorylation (Hamajima et al., 2009). Accordingly, increased phosphorylation at the linker region of Smad3 in the hepatocytes adjacent to the fibrous area in livers infected with chronic hepatitis C has been observed, thus suggesting a role for the ECM on the linker phosphorylation of Smad3 (Matsuzaki et al., 2007).

When integrins attenuate the activation of TGF-β1-induced apoptosis, the expression of PDCD4, a pro-apoptotic molecule downstream of TGF-β1, is repressed (unpublished data). ERK and JNK inhibitors, but not a p38 inhibitor, restored the formation of pSmad2C. All three inhibitors restored pSmad3C formation, and the transfection of a linker region mutated Smad3, but not Smad2, restored the TGF-β1-induced transcriptional activity, suggesting the differential roles of Smad2 and Smad3 (Hamajima et al., 2009; Matsuzaki, 2011).

Future perspectives for prevention and therapy

Recent studies have shown that the phosphorylation state of the Smad TFs plays a pivotal role in converting the tumor suppressor signal of TGF-β1 to a tumor promoting signal. Thus, the inhibition of the linker phosphorylation of Smads could be an attractive approach to inhibit the development and progression of HCC. Among the kinases that phosphorylate the linker region of Smads directly or indirectly, JNK has been shown to phosphorylate the linker regions of both Smad2 and Smad3. Indeed, administration of a JNK inhibitor to rats successfully inhibited the development of HCC in a DEN-induced HCC model (Nagata et al., 2009). Integrins can propagate the multiple signals that promote the survival and growth of HCC cells by interacting with growth factor receptors, including the TGF-β receptor. Therefore inhibitors of integrin activation, which might modulate the phosphorylation

![Diagram](https://www.frontiersin.org/)

**Figure 4** The interactions between TGF-β1 and ECM/integrin-mediated signaling in hepatocytes in the fibrotic liver. Initially, TGF-β1 inhibits the growth of hepatocytes by inducing the expression of pro-apoptotic molecules, such as PDCD4, via the formation of pSmadC. TGF-β1 stimulates the production of fibrous ECM from activated HSCs/myofibroblasts. The accumulated ECM proteins in turn activate integrins, then these activated integrins and co-existing growth factors inhibit the TGF-β1-induced growth-suppressive signals via the formation of pSmadL, leading to the resistance of hepatocytes to apoptosis, and the promotion of hepatocarcinogenesis.
of Smad indirectly, could represent an alternative approach for the prevention and/or treatment of HCC and tumor progression. In fact, antisense-mediated inhibition of integrin αv or integrin β3 suppressed the growth of HCC cells injected subcutaneously in a mouse model (Li et al., 2007). Various types of integrin inhibitors are currently being examined in preclinical and clinical studies (Desgrosellier and Cheresh, 2010; Millard et al., 2011). Further studies are necessary to clarify the interaction between integrins and TGF-β1, and will contribute to the development of novel therapeutics for HCC.

REFERENCES

Alam, N., Goel, H. L., Zarif, M. J., Butterfield, J. E., Perkins, H. M., Sansoucy, B. G., Sawyer, T. K., and Languino, L. R. (2007). The integrin–growth factor receptor duet. J. Cell Physiol. 213, 649–653.

Baron, V., and Schwartz, M. (2000). Cell adhesion regulates ubiquitin-mediated degradation of the platelet-derived growth factor receptor beta. J. Biol. Chem. 275, 39318–39323.

Bedossa, P., Peltier, E., Terris, B., Franco, D., and Polyanrd, T. (1995). Transforming growth factor-beta 1 (TGF-beta 1) and TGF-beta 1 receptors in normal, cirrhotic and neoplastic human livers. Hepatology 21, 760–765.

Begum, N. A., Mori, M., Matsumata, T., Takenaka, K., Sugimachi, K., and Barnard, G. F. (1995). Differential display and integrin alpha 6 mes- senger RNA overexpression in hepato- cellular carcinoma. Hepatology 22, 1447–1455.

Bergamini, C., Sgarra, C., Terotoli, P., Lupo, L., Azzariti, A., Antonaci, S., and Giannelli G. (2007). Laminin-5 stimulates hepatocellular carci- noma growth through a differ- ent function of alpha6beta4 and alpha3beta1 integrins. Hepatol. 46, 1801–1809.

Bhowmick, N. A., Ghiassi, M., Bakin, A., Ahsan, M., Lundquist, C. A., Angui, M. G., Perkins, H. M., and Moses, H. L. (2001). Transforming growth factor-beta mediates epithelial to mesenchymal trans- differentiation through a RhoA- dependent mechanism. Mol. Biol. Cell 12, 27–36.

Bierie, B., and Moses, H. L. (2006). TGFβ: the molecular jekyll and Hyde of cancer. Nat. Rev. Cancer 6, 506–520.

Bottcher, R. T., Lange, A., and Fassler, R. (2009). How ILK and kindlins cooperate to orchestrate integrin sig- naling. Curr. Opin. Cell Biol. 21, 670–675.

Burch, M. L., Zheng, W., and Little, P. J. (2011). Smad linker phosphoryl- ization in the regulation of extracellular matrix synthesis. Cell Mol. Life Sci. 68, 97–107.

Cabodi, S., Moro, L., Bergatto, E., Boeri Erba, E., Di Stefano, P., Turco, E., Tarone, G., and De Filippis, P. (2004). Integrin regulation of epidermal growth factor (EGF) receptor and of EGF-dependent responses. Biochem. Soc. Trans. 32, 438–442.

Caja, L., Ortiz, C., Bertran, E., Murillo, M. M., Miró-Obradors, M. J., Palacios, E., and Fabregat, I. (2007). Differential intracellular signaling induced by TGF-beta in rat adult hepatocytes and hepatoma cell lines: implications in liver carcinogenesis. Cell. Signal. 19, 683–694.

Carloni, V., Mazzocca, A., Pantaleo, P., Cordella, C., Lafi, G., and Gentilini, P. (2001). The integrin, alphabeta1, is necessary for the matrix-dependent activation of FAK and MAP kinase and the migration of human hepatocarcinoma cells. Hepatology 34, 42–49.

Coulaveld, A., Sozay, J.-Y., and Feldmann, G. (1993). Expression of cell- cell and cell-matrix adhesion pro- teins by sinusoidal endothelial cells in the normal and cirrhotic human liver. Am. J. Pathol. 143, 738–752.

Cukierman, E., Pankov, R., Stevens, D., and Yamada, K. M. (2008). Taking cell-matrix adhesions to the third dimension. Science 294, 1708–1712.

Derynck, R., and Zhang, Y. (2003). Smad-dependent and Smad- independent pathways in TGF-beta family signaling. Nature 425, 577–584.

Desgrosellier, J. S., and Cheresh, D. A. (2010). Integrins in cancer: their genetic, epigenetic, and therapeutic opportunities. Nat. Rev. Cancer 10, 9–22.

El-Serag, H. B., and Rudolph, K. L. (1996). Epidermal growth factor, but not transforming growth factor-beta1 mediates growth by integrin-growth factor receptor duet. J. Biol. Chem. 275, 163–180.

El-Serag, H. B., and Rudolph, K. L. (1998). Long-term expression of different- ized functions in hepatocytes cultured in three-dimensional col- lagen matrix. J. Cell. Physiol. 175, 553–562.

Hamajima, H., Ozaki, I., Zhang, H., Iwane, S., Kawaguchi, Y., Eguchi, Y., Yoshizaki, H., Mizuta, T., Mat- szuksi, K., Fujimoto, K. (2009). Modulation of the transforming growth factor-beta1-induced Smad phosphorylation by the extracellular matrix receptor beta1-integrin. Int. J. Oncol. 35, 1441–1447.

Hansen, L. M., and Giannelli G. (2007). Laminin-5 stimulates hepatocellular carcinoma development using liver stiffness measurement (FibroScan). Hepatology 53, 885–894.

Iwane, S., Kawaguchi, Y., Eguchi, Y., Yoshizaki, H., Mizuta, T., Mat- szuksi, K., Fujimoto, K. (2009). Modulation of the transforming growth factor-beta1-induced Smad phosphorylation by the extracellular matrix receptor beta1-integrin. Int. J. Oncol. 35, 1441–1447.

Hansen, L. M., and Giannelli G. (2007). Laminin-5 stimulates hepatocellular carcinoma development using liver stiffness measurement (FibroScan). Hepatology 53, 885–894.

Kamaraju, A. K., and Roberts, A. B. (2005). Role of Rho/ROCK and p38 MAP kinase pathways in transforming growth factor-beta-mediated Smad-dependent growth inhibition of human breast carcino- noma cells in vivo. J. Biol. Chem. 280, 1024–1036.

Ke, A. W., Shi, G. M., Zhou, J., Huang, X. Y., Shi, Y. H., Ding, Z. B., Wang, X. Y., Devbhandari, R. P., and Fan, J. (2011). CD151 amplifies signal- ing by integrin αvβ3 to PDG and induces the epithelial–mesenchymal transition in HCC cells. Gastroenterology 140, 1629–1641.

Kretzschmar, M., Doody, J., Timokhina, I., and Massagué, J. (1999). A mech- anism of repression of TGF-β/Smad signaling by oncogenic Ras. Genes Dev. 13, 804–816.
with hepatitis C virus infection per-
turbs hepatic transforming growth factor beta signaling, promoting carcinoma. Hepatology 46, 48–57.
Millard, M., Odde, S., and Neamati, N. (2011). Integrin targeted therapeuticstherapies. Theranostics 1, 154–188.
Mori, S., Matsuzaki, K., Yoshida, K., Furukawa, F., Tahashi, Y., Yamagata, H., Sekimoto, G., Seki, T., Matsui, H., Nishizawa, M., Fujisawa, J., and Okazaki, K. (2004). TGF-beta and HGF transmit the signals through the JNK-dependent Smad2/3 phosphorylation at linker regions. Oncogene 23, 7416–7429.
Morlo, L., Dolce, L., Cabodi, S., Bergatto, E., Boeri-Erba, E., Smeriglio, M., Turco, E., Retta, S. F., Guiffrida, M., Montuori, M., Godovac-Zimmermann, J., Conti, A., Schaefer, E., Beguinot, L., Tacchetti, C., Gaggin, P., Silengo, L., Taroni, G., and Defilippi, P. (2002). Integrin-induced epithelial growth factor (EGF) receptor activation requires the Src and p130Cas and leads to phosphorylation of specific EGF receptor tyrosines. J. Biol. Chem. 277, 9405–9414.
Moses, H. L., Yang, E. Y., and Pietten-
pol, J. A. (1990). TGF-ß stimulation and inhibition of cell proliferation: new mechanistic insights. Cell 63, 245–249.
Moustakas, A., and Heldin, C. H. (2005). Non-Smad TGF-beta signals. J. Cell Sci. 118, 3573–3584.
Murata, M., Matsuzaki, K., Yoshida, K., Sekimoto, G., Tahashi, Y., Mori, S., Uemura, Y., Sakaia, N., Fujisawa, J., Seki, T., Kobayashi, K., Yokote, K., Koike, K. and Okazaki K. (2009). Hepatitis B virus X protein shifts human hepatic transforming growth factor (TGF)-beta signaling from tumor suppression to oncogenesis in early chronic hepatitis B. Hepatology 49, 1203–1217.
Murillo, M. M., del Castillo, G., Sánchez, A., Fernández, M., and Fabregat, I. (2005). Involvement of EGF receptor and c-Src in the survival signals induced by TGF-beta in hepatocytes. Oncogene 24, 4580–4587.
Nagata, H., Hatano, E., Tada, M., Murata, M., Kitamura, K., Asechi, H., Narita, M., Tanaki, N., Yagi, S., Ikai, I., Matsuzaki, K., and Uemoto, S. (2009). Inhibition of c-Jun NH2-terminal kinase switches Smad3 signaling from oncogene to tumor-suppression in rat hepato-
tocellular carcinoma. Hepatology 49, 1944–1953.
Nakashima, M., Hamajima, H., Xia, J., Iwane, S., Kwaguchy, Y., Eguchi, Y., Mizuta, T., Fujimoto, K., Ozaki, L., and Matsuhashi, S. (2010). Regulation of tumor suppressor PDCD4 by novel protein kinase C iso-
forms. Biochem. Biophys. Acta 1803, 1020–1027.
Oberhammer, F. A., Pavelka, M., Sharma, S., Tiefenbacher, R., Purcho, A. F., Bursch, W., and Shul
te-Hermann, R. (1992). Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor beta 1. Proc. Natl. Acad. Sci. U.S.A. 89, 5408–5412.
Okuda, K. (2000). Hepatocellular carci-
noma. J. Hepatol. 32, 225–257.
Ozaki, I., Yamamoto, K., Mizuta, T., Kajihara, S., Fukushima, S., Setoguchi, Y., Morito, F., and Sakai, T. (1998). Differential expression of laminin receptors in human hepatocellular carcinoma. Gut 43, 837–842.
Perlman, R., Schiennmann, W. P., Brooks, M. W., Lodish, H. F., and Weinberg, R. A. (2001). TGF-beta-induced apoptosis is mediated by the adapter protein Daxx that facilitates JNK activation. Nat. Cell Biol. 3, 708–714.
Petríčková, C., Beug, H., Balmain, A., and Ofi, M. (2000). TGF-beta inhibits p70 S6 kinase via protein phos-
phatase 2A to induce G(1) arrest. Genes Dev. 14, 3093–3101.
Reginato, M. J., Mills, K. R., Paulus, J. K., Lynch, D. K., Sgroi, D. C., Debnath, J., Muthuswamy, S. K., and Brugge, J. S. (2003). Integrins and EGFR coordinately regulate the pro-apoptotic protein Bim to prevent anoikis. Nat. Cell Biol. 5, 733–740.
Sakaida, I., Hironakà, K., Uchida, K., Suzuki, C., Kayano, K., and Okita, K. (1998). Fibrosis accelerates the development of enzyme-altered lesions in rat liver. Hepatology 28, 1247–1252.
Schrader, J., Gordon-Walker, T. T., Aucott, R. L., van Deemter, M., Ates, M., Kajihara, S., Fukushima, N., Oberhammer, F. A., Pavelka, M., Smeriglio, M., E., Boeri-Erba, E., Godovac-Zimmerman, J., Conti, A., Schaefer, E., Beguinot, L., Tacchetti, C., Gaggin, P., Silengo, L., Taroni, G., and Defilippi, P. (2002). Integrin-


Volpes, R., van den Oord, J. J., and Desmet, V. J. (1993). Integrons as differential cell lineage markers of primary liver tumors. Am. J. Pathol. 142, 1483–1492.

Wang, R., Ferrell, L. D., Faouzi, S., Maher, J. J., and Bishop, J. M. (2001). Activation of the Met receptor by cell attachment induces and sustains hepatocellular carcinomas in transgenic mice. J. Cell Biol. 153, 1023–1034.

Wells, R. G. (2008). The role of matrix stiffness in regulating cell behavior. Hepatology 47, 1394–1400.

Wrighton, K. H., Lin, X., and Fen, X. (2009). Phospho-control of TGF-β superfamily signaling. Cell Res. 19, 8–20.

Yang, Y. A., Zhang, G. M., Feigenbaum, L., and Zhang, Y. E. (2006). Smad3 reduces susceptibility to hepatocarcinoma by sensitizing hepatocytes to apoptosis through downregulation of Bcl-2. Cancer Cell 9, 445–457.

Yi, J. Y., Shin, I., and Arteaga, C. L. (2005). Type 1 transforming growth factor-beta receptor binds to and activates phosphatidylinositol 3-kinase. J. Biol. Chem. 280, 10870–10876.

Yoo, J., Ghiassi, M., Jirmanova, L., Baleit, A. G., Hoffman, B., Fornace, A. J. Jr., Liebermann, D. A., Bottinger, E. P., and Roberts, A. B. (2003). Transforming growth factor-beta-induced apoptosis is mediated by Smad-dependent expression of GADD45β through p38 activation. J. Biol. Chem. 278, 43001–43007.

Yoshida, H., Shiratori, Y., Miziyama, M., Arakawa, Y., Ide, T., Sata, M., Inoue, O., Yano, M., Tanaka, M., Fujiyama, S., Nishiguchi, S., Kuroki, T., Imazeki, F., Yokosuka, O., Kinoyama, S., Yamada, G., and Omata, M. (1999). Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and non-cirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by interferon therapy. Ann. Intern. Med. 131, 174–181.

Yoshida, K., Matsuzaki, K., Mori, S., Tahashi, Y., Yamagata, H., Furukawa, F., Seki, T., Nishizawa, M., Fujisawa, J., and Okazaki, K. (2005). Transforming growth factor-beta and platelet-derived growth factor signal via c-Jun N-terminal kinase-dependent Smad2/3 phosphorylation in rat hepatic stellate cells after acute liver injury. Am. J. Pathol. 166, 1029–1039.

Zhang, H., Ozaki, I., Mizuta, T., Yoshimura, T., Matsuhashi, S., Eguchi, Y., Yasutake, T., Hisatomi, A., Sakai, T., and Yamamoto, K. (2003). Mechanism of beta 1-integrin-mediated hepatoma cell growth involves p27 and S-phase kinase-associated protein 2. Hepatology 38, 305–313.

Zhang, H., Ozaki, I., Mizuta, T., Yoshimura, T., Matsuhashi, S., Eguchi, Y., Yasutake, T., Hisatomi, A., Sakai, T., and Yamamoto, K. (2004). Transforming growth factor-beta 1-induced apoptosis is blocked by beta 1-integrin-mediated mitogen-activated protein kinase activation in human hepatoma cells. Cancer Sci. 95, 878–886.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 12 August 2011; paper pending published: 06 September 2011; accepted: 11 October 2011; published online: 24 October 2011.

Citation: Ozaki I, Hamajima H, Matsushashi S and Mizuta T (2011) Regulation of TGF-β1-induced pro-apoptotic signaling by growth factor receptors and extracellular matrix receptor integrins in the liver. Front. Physio. 2, 78. doi: 10.3389/fphys.2011.00078

This article was submitted to Frontiers in Gastrointestinal Sciences, a specialty of Frontiers in Physiology.

Copyright © 2011 Ozaki, Hamajima, Matsushashi and Mizuta. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.