Growth Factors and Regeneration

Digestive Diseases

Dig Dis 2012;30:514–523
DOI: 10.1159/000341704

TGF-β Signaling in Onset and Progression of Hepatocellular Carcinoma

Nadja M. Meindl-Beinker[a] Koichi Matsuzaki[b] Steven Dooley[a]

[a]Molecular Hepatology – Alcohol-Associated Diseases, II. Medical Clinic, Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany; [b]Department of Gastroenterology and Hepatology, Kansai Medical University, Moriguchi, Japan

Key Words
TGF-β · Hepatocellular carcinoma · Liver disease

Abstract
Transforming growth factor (TGF)-β is a central regulator in chronic liver disease, contributing to all stages of disease progression from initial liver injury through inflammation and fibrosis to cirrhosis and hepatocellular carcinoma. Liver damage-induced levels of active TGF-β enhance hepatocyte destruction and mediate hepatic stellate cell and fibroblast activation resulting in a wound-healing response, including myofibroblast generation and extracellular matrix deposition. Further evidence points to a decisive role of cytostatic and apoptotic functions mediated on hepatocytes, which is critical for the control of liver mass, with loss of TGF-β activities resulting in hyperproliferative disorders and cancer. This concept is based on studies that describe a bipartite role of TGF-β with tumor suppressor functions at early stages of liver damage and regeneration, whereas during cancer progression TGF-β may turn from a tumor suppressor into a tumor promoter that exacerbates invasive and metastatic behavior. We have delineated this molecular switch of the pathway from cytostatic to tumor promoting in further detail and identify activation of survival signaling pathways in hepatocytes as a most critical requirement. Targeting the TGF-β signaling pathway has been explored to inhibit liver disease progression. While interfering with TGF-β signaling in various short-term animal models has demonstrated promising results, liver disease progression in humans is a process of decades with different phases in which TGF-β or its targeting may have both beneficial and adverse outcomes. We emphasize that, in order to achieve therapeutic effects, targeting TGF-β signaling in the right cell type at the right time is required.

Transforming growth factor (TGF)-β is a multiplicity factor mediating cellular processes, including cell growth, cell differentiation, apoptosis, and cellular homeostasis. The canonical TGF-β signaling pathway starts with binding of the ligand to the type II receptor, which is subsequently activated by autophosphorylation and recruits the type I receptor ALK-5. In the next step, the type I receptor is activated by phosphorylation and this active ligand receptor complex then induces association of the intracellular signal mediators, the receptor (R)-Smad proteins 2 and 3. R-Smads are activated by the receptor complex through phosphorylation and form a complex

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0257–2753/12/0305–0514$38.00/0

Accessible online at:
www.karger.com/ddi

Prof. Dr. Steven Dooley
Molecular Hepatology – Alcohol-Associated Diseases
II. Medical Clinic, Medical Faculty Mannheim of Heidelberg University
Theodor-Kutzer-Ufer 1–3, DE–68167 Mannheim (Germany)
Tel. +49 621 383 3768, E-Mail Steven.Dooley@medma.uni-heidelberg.de

with the common mediator (Co)-Smad4, which increases the retention time of the principally back and forth shuttling R- and Co-Smad proteins in the nucleus, thereby facilitating transcriptional regulation of target genes. Besides this canonical signaling pathway, TGF-β is able to transduce its signal via a variety of other downstream routes. In some cell types including hepatic stellate cells (HSC) and hepatocytes in the liver, instead of ALK-5, TGF-β may also signal via the type I receptor ALK-1, thereby activating R-Smads 1, 5, and 8. This then leads to a different set of TGF-β-regulated target genes, as compared to the TGF-β/ALK-5 signaling pathway. Moreover, the activated TGF-β receptor complex, besides the Smads, may use a different set of intracellular signaling mediators, e.g. MAP kinase signaling pathway components may be used from TGF-β to translate its action into the nucleus [1]. This not being enough, activated R-Smad proteins may in addition form heteromeric complexes with components of other signaling pathways, e.g. with phosphorylated STAT3 from the JAK/STAT signaling pathway, thereby further increasing the repertoire of potentially TGF-β-regulated target genes [2]. Finally, R-Smad proteins may be direct substrates of signaling pathways other than TGF-β, which of course induces a different outcome in the cell. One example is the c-Jun N-terminal kinase (JNK) signaling cascade that mediates R-Smad linker phosphorylation instead of the ALK-5-dependent canonical C-terminal phosphorylation [3], thereby enabling a dramatic switch with regard to the cellular fate as will be discussed below.

A third group of Smad proteins comprises the inhibitory Smads, Smad6 and Smad7 [4]. Smad6 is specific for interference with the R-Smad1, 5, and 8 pathway, whereas overexpression of Smad7 in a cell completely shuts off R-Smad-dependent TGF-β signaling very efficiently. Besides this variability in TGF-β downstream signaling, a cohort of components have been described that may modulate the different steps of the signaling pathway from the cell surface to the nucleus, comprising, among other things, receptor complex formation, apical-basal polarity, trafficking, signaling crosstalk, binding partners of R- and Co-Smad proteins, transcriptional cofactors, and DNA binding partners. Taylor and Wrana [5] gave an overview of those in a snapshot of the TGF-β pathway interactome, therewith visualizing very impressively the complexity of TGF-β signaling.

Not only due to the above, the TGF-β signaling outcome is versatile and cell type dependent. In a given tissue, TGF-β may impact nearly every cell that comes in contact with it. Regarding epithelia, TGF-β may induce cell cycle arrest, apoptosis, and adhesion, or extracellular matrix and cytokine production. In fibroblasts, instead, TGF-β induces proliferation and stimulates ECM production and cytokine secretion. Regarding the endothelium, TGF-β upregulates migration and morphogenesis or controls proliferation and growth, whereas in immune cells TGF-β is anti-inflammatory and interferes with T-cell proliferation, NK-cell function, and antigen presentation [6].

In normal liver, usually no active TGF-β is found, whereas upon acute damage and during chronic liver disease progression TGF-β levels are rapidly induced, continuously increasing with disease severity. This can be monitored in the serum of patients and therefore represents one promising and frequently used parameter for diagnosing liver disease in the serum of patients [7, 8].

The best studied liver cell with regard to the outcome of TGF-β is the HSC. In this cell type, TGF-β represents the most important factor for activation and myofibroblast transdifferentiation. If TGF-β signaling is blocked in stellate cells during a damage situation, myofibroblast generation and fibrogenesis are blunted. Typical features of myofibroblasts in the setting of fibrosis are upregulated expression of α-smooth muscle actin (αSMA), TGF-β, PDGF, connective tissue growth factor (CTGF), type I collagens, tissue inhibitor of metalloproteinase 1 (TIMP1), and other extracellular matrix proteins. Further features of HSC activation comprise gain of proliferation, migration, and contractility, along with loss of vitamin A droplet storage [9].

Cytostatic action of TGF-β on proliferating hepatocytes during liver regeneration was identified a long time ago. For example, Braun et al. [10] reported already in 1988 that TGF-β mRNA increases during liver regeneration, therewith identifying a possible paracrine mechanism of growth regulation. In animal models of liver regeneration using partial hepatectomy, increased TGF-β levels were identified shortly after proliferation peaks of hepatocytes and biliary ductular cells and in parallel with the proliferation peaks of HSC and Kupffer cells [11]. Mainly from such data it is suggested that TGF-β acts cytostatically on proliferating epithelial cells during liver regeneration, although the molecular details and downstream signaling pathways have not been investigated in detail. A summary of the TGF-β role in liver regeneration can be found in the report by Karkampouna et al. [8].

When studying downstream signaling arms of the TGF-β pathway more thoroughly, different branches can be discriminated [12]. There are on one hand the Smad-

mediated gene responses in the nucleus, which can be divided into two major arms. One comprises its function as a tumor suppressor, including cytostasis (e.g. cyclin-dependent kinase – CDK – and c-myc inhibition), differentiation (e.g. ID1 regulation), and apoptosis (e.g. downregulation of BCL-2 and upregulation of BIM and GADD45β). The other Smad-directed arm relates to effects like phenotypic plasticity (e.g. epithelial mesenchymal transition inducers) and environmental components (e.g. extracellular matrix proteins, cytokines, and proteases) or has impact on signaling pathways due to regulation of receptor, signal transducer, and transcription factor expression. Besides these gene responses, there are also TGF-β-mediated stress responses. Here, the relations with JNK/p38, Cdc42/Rho, and Par6 were described, which directly link TGF-β activated receptor complexes with an impact on cell migration, cell shape, and cell-cell contacts [12].

In early steps of carcinogenesis, epithelial cells may evade the tumor-suppressive action of TGF-β. This may principally be achieved via two different routes. On the one hand, tumor suppression can be disabled by mutation of pathway core components. This has been described for the TGF-β type I receptor, e.g. in ovary, esophagus, and head and neck cancer, and for the TGF-β type II receptor, e.g. in colon, stomach, biliary, lung, and ovarian cancer. Also, loss of functional Smads 2, 3, and 4 via mutation or genetic losses has been demonstrated in pancreatic, colon, and esophagus cancer. In those cases, due to total loss of functional signaling components, no or only a few TGF-β responses remain for the cell, e.g. Smad4-independent responses in pancreatic cancers with Smad4 loss-of-function mutations. On the other hand, tumor suppression can be disabled without such loss-of-function mutations/genomic alterations by selective inhibition of the aforementioned tumor suppressor/cytostatic arm. This was shown, for example, in glioma due to p15/INK4B deletion or through FoxO inhibition by Akt, or in breast cancer by LIP-dependent C/EBPβ inhibition and/or an ID1 switched response. In such settings, the TGF-β signaling components are fully available, but their downstream signaling leading to cytostatic responses is amputated, whereby TGF-β downstream signaling is redirected towards gene responses that facilitate tumor progression and metastasis. TGF-β signaling then may lead to phenotypic plasticity (HMGA2, snail, and ID1 are corresponding signaling targets) or to the production of components that have a major impact on the environment (ANGPTL4, CTGF, IL1, PthRP, PDGF, and VEGF) [12].

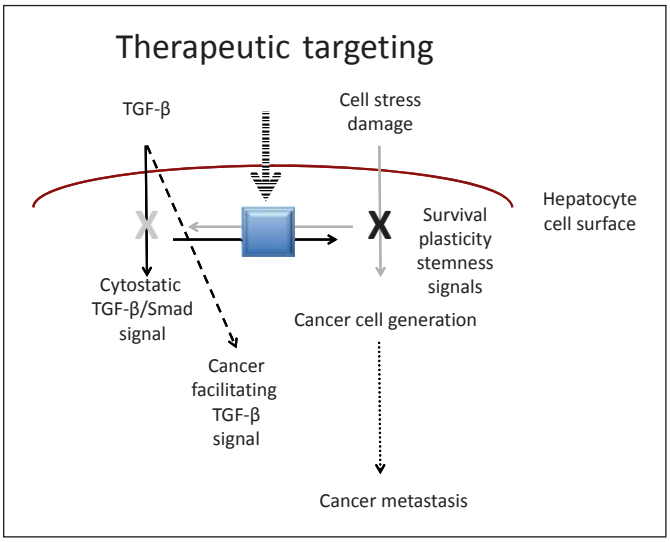

Fig. 1. In liver homeostasis, TGF-β provides a cytostatic signal mediating proliferation inhibition, apoptosis, and growth control (left arrow). This setting is tightly regulated by signaling pathways inducing cell survival. Upon cell stress and damage, e.g. mediated by hepatitis viral infection, drug detoxification, or others, survival signaling is initiated and hepatocytes are prepared to undergo morphological changes, including plasticity and expression of stemness factors (right arrow). This abrogates the cytostatic TGF-β signal and redirects the pathway to a cancer-facilitating signature (dashed arrow). Survival and stemness signals with plasticity and tumorigenic TGF-β signals facilitate cancer cell generation and malignant progression (dotted arrow). Based on this model of hepatocellular carcinogenesis, we hypothesize that a combined therapeutic approach interfering with survival signaling, e.g. inhibiting pAKT plus stimulation of cytostatic TGF-β signaling, is promising in the early stages of malignant transformation (middle arrow and square switch).

To delineate the switch of TGF-β effects from wound healing to tumor promotion during HCC development, one first has to understand the dynamics of histopathological progression and molecular features of HCC development upon hepatic injury. It can be incurred by anyone of several factors including, among others, hepatitis B virus or hepatitis C virus infection, alcohol, and α-toxin B1, and upon initiation there is hepatocyte damage and necrosis followed by hepatocyte proliferation and liver regeneration. Continuous cycles of destructive and regenerative processes foster a chronic liver disease condition that culminates in liver cirrhosis. Cirrhosis is characterized by macroregenerative nodules that, due to increased proliferative activity, are surrounded by collagen deposition and scarring. In the next progression stage, hyper-

plastic nodules are observed, followed by dysplastic nodules and ultimately HCC, which can be further classified into well-differentiated, moderately differentiated, and poorly differentiated tumors, with the latter ones representing the most malignant form of primary HCC. Late disease stages include marked genomic instability and loss of p53 function. A summary of the different stages from liver damage to HCC can be found in the report by Farazi and DePinho [13].

One molecular mechanism for how liver cells can escape from the cytostatic TGF-β response was described by the work of Mishra and coworkers [14–24]. Initially, they found that disruption of the adaptor protein ELF, a β-spectrin, leads to disruption of TGF-β/Smad signaling. Furthermore, Elf–/– mice displayed a phenotype similar to Smad2+/– and Smad3+/– mice with midgestational death due to gastrointestinal, liver, neural, and heart defects. TGF-β triggers phosphorylation and association of ELF with Smad3 and Smad4, followed by nuclear translocation, therewith indicating an unexpected molecular link between a scaffolding protein and the TGF-β signaling pathway [24]. ELF-mediated TGF-β-dependent transcriptional responses are strongly related to its cytostatic response, comprising, for example, induction of p15, p21, MMP-9, p57, β-cadherin, and p16 expression as well as repression of β-catenin, c-myc, hTERT, IGF2, and LDLR transcription [14, 18, 20, 21]. Additionally, PRAJA, a RING-H2 protein, was identified to interact with ELF leading to enhanced degradation via substantial E3-dependent ubiquitinylation. Thus, high-level expression of PRAJA may interfere with cytostatic TGF-β effects by reducing the availability of ELF for efficient R-Smad signal propagation [22]. Such data could also be validated by correlative immunohistochemical stainings in human HCC patients.

Mishra and coworkers further pointed out that up to 40% of hepatocellular carcinomas (HCC) are clonal and potentially arise from cancer stem cells (CSCs), characterized by expression of stem cell markers like Oct4, Nanog, or Sox2, increased activation of multiple pathways including IL-6/STAT3, WNT, CDK4, and hedgehog, and a parallel loss of the TGF-β cytostatic response [23].

From these findings and from work with cultured HCC cells, it is concluded that in a setting of low ELF and TβRII expression or upregulated PRAJA expression, Smad3-mediated cytostatic TGF-β signaling is disrupted. Parallel activation of IL6, hedgehog, Notch, and Wnt signaling pathways will then facilitate the generation of CSCs that may reflect the source of HCC development. It is further suggested that modulating stem cell renewal factors such as STAT3, NANOG, and OCT4 may reduce HCC formation [25].

To prove the hypothesis of Mishra, we investigated ten different human HCC lines (manuscript in preparation). Measuring cytotoxicity, proliferation, Smad-binding element reporter activation and expression of ELF and PRAJA, it was obvious that Hep3B and HuH7 displayed a significant cytostatic response as well as Smad3/4-binding element reporter activation upon TGF-β treatment, accompanied by high ELF and low PRAJA expression. Vice versa, most of the cell lines that were not responsive to a cytostatic TGF-β signal had low reporter gene activation, very low ELF expression, and/or upregulated PRAJA expression (HCC-M, HCC-T, HepG2, and HuH6). Interestingly, FLC-4 cells which had also lost the TGF-β cytostatic response displayed quite high levels of ELF but at the same time strongly upregulated PRAJA expression. These findings in cell lines at least partially confirm a mechanism as described by Mishra and coworkers, indicating a significant correlation of TGF-β Smad-mediated cytostatic signaling and ELF expression (manuscript in preparation).

In another very interesting study, the Machida lab very impressively demonstrated the connection of hepatitis C virus infection, alcohol intoxication, CSC generation, and HCC development. Their experiments illustrated that HCV infection induces TLR-4 expression in hepatocytes, therewith sensitizing this cell type to the direct action of lipopolysaccharides (LPS), which are available in increased concentrations in the blood and liver upon alcohol consumption. Continuous LPS-TLR4 signal transduction in hepatocytes induces expression of Nanog, a stem cell marker, and facilitates the formation of HCC [26]. In a next step, CSCs were purified from such HCC tumors and a liver CSC expression library was generated. In a functional screen using a p53-deficient CSC line and a soft agar colony formation assay, STAT3, YAP1, and Igf2bp3 were identified as proto-oncogenes in HCV/alcohol-mediated liver cancer. In a large series of in vivo and in vitro experiments, Machida and coworkers could delineate the mechanism responsible for the oncogenic activities of Nanog-dependent CSCs. Based on knowledge of a previous finding that YAP is able to stabilize Smad7 [27], a strong inhibitor of the TGF-β/Smad signaling pathway, they were able to connect the oncogenic action of Yap1 with cytostatic TGF-β signaling. In their investigation, they could show that TGF-β signaling was interfered with two-fold: (i) by increasing the negative regulatory activity of Smad7 through YAP1 and (ii) by Igf2bp3-

mediated activation of Akt survival signaling, which induces mTOR activity to interfere with Smad3-mediated TGF-β signaling. In the aforementioned setting, cancer cells are rather resistant to chemotherapy, e.g. rapamycin has only a limited inhibitory effect on mTOR, whereas sorafenib-induced cell death seems to require functional TGF-β cytostatic signaling and is also abrogated in cancer cells during ongoing survival signaling. This was confirmed in functional experiments upon silencing of YAP1 and Igf2bp3, where survival signaling in CSCs was blocked and cells were resensitized to cytostatic TGF-β signaling. Moreover, such cancer cells were responsive for the action of rapamycin and sorafenib, which were able to significantly induce cell death. In conclusion, Machida and coworkers demonstrated a causal link between pro-oncogenic TLR-4 signaling and a defective TGF-β tumor suppressor pathway, which is mediated by the TLR-4/Nanog target genes Yap1 and Igf2bp3 (pers. commun.; manuscript in preparation, and a report by Machida et al. [28]).

In our studies with HCC cell lines, we could also find supportive data for this study. We found a negative correlation between intrinsic Smad7 expression and the duration of Smad2 activation. Cell lines with low Smad7 expression display a prolonged phosphorylated (p) Smad2 signal as compared to such cell lines with high intrinsic Smad7 expression, which have only a transient upregulation and subsequent degradation of pSmad2. On the other hand, Smad7 expression was nicely correlated with TGF-β expression of the respective cell lines. Given that TGF-β may induce Smad7 expression, the identified profile may reflect autocrine TGF-β effects. The most significant support for the notion that Smad7 overexpression may indeed participate in liver carcinogenesis came from studying Smad7 mRNA expression in 146 matched HCC/normal liver tissue samples, where 65.8% displayed Smad7 overexpression in cancer cells (manuscript in preparation). To obtain further insight into the role of TGF-β/Smad7 in liver cancer, we are currently investigating mouse strains with hepatocyte-specific and inducible Smad7 overexpression/knockout in a model of liver cancer using DEN treatment and we are investigating strains generated by crossing those animal models with FAH–/– mice, which spontaneously develop HCC.

The lab of Matsuzaki identified an important molecular switch mechanism from TGF-β cytostatic towards fibrogenic/carcinogenic action that is directly linked to biochemical modulations of Smad2 and Smad3. They could show that the canonical signaling pathway comprising binding of TGF-β to TβRII/ALK5 leads to direct phosphorylation of the C-terminus of Smad3 (pSmad3C) in hepatocytes, which subsequently inhibits hepatocyte proliferation by upregulating $p21^{WAF1}$ transcription. Stress situations towards hepatocytes, e.g. hepatitis virus infections, pro-inflammatory cytokines, alcohol, drug intoxication, and somatic mutations, can transmit mitogenic signals, among others, through the JNK-dependent signaling pathway. Activated JNK is able to phosphorylate Smad3 at the linker region (pSmad3L) and thus initiates the pSmad3L pathway leading to hepatocyte proliferation, possibly by stimulating transcription of the c-Myc gene. Additionally, linker phosphorylation of Smad3 indirectly prevents Smad3 C-terminal phosphorylation, pSmad3C-mediated $p21^{WAF1}$ transcription, and consequently the cytostatic effect of TGF-β in normal hepatocytes. Either various JNK inhibitors or a Smad3 mutant lacking the JNK phosphorylation site in the linker region can eliminate mitogenic pSmad3L signaling and therewith restore the loss of pSmad3C signaling observed in normal hepatocytes [29–31]. On the other hand, collagen synthesis by mesenchymal cells including fibroblasts, mesangial cells, and HSC appears to be promoted by the dually phosphorylated Smad2 (pSmad2L/C) pathway. Thus, linker phosphorylation of Smad3 antagonizes its C-terminal phosphorylation, but linker and C-terminal phosphorylation of Smad2 act synergistically in driving fibrogenesis. In patients with HCV-related chronic liver diseases, chronic inflammation shifts hepatocytic pSmad signaling from tumor-suppressive pSmad3C to carcinogenic pSmad3L and fibrogenic pSmad2L/C branches, which accelerates liver fibrosis and increases the risk of HCC. Chronic inflammation caused by HCV infection thereby represents an early fibro-carcinogenic step, providing a nonmutagenic tumor-promoting stimulus. In advanced fibrotic livers, mitogenic, genetic, or epigenetic alterations drive multi-step fibro-carcinogenesis. Patients with mild fibrosis respond effectively to anti-viral therapy. According to the hypothesis of Matsuzaki, this is at least partially based on successful switching of pSmad signaling from carcinogenic pSmad3L and fibrogenic pSmad2L/C to tumor-suppressive pSmad3C. That means that if patients achieve anti-viral treatment before hepatocytes have acquired oncogenic potential, HCV clearance interferes with fibrosis and reduces HCC incidence. However, HCC develops particularly in patients with advanced liver fibrosis, where an inflammation-independent process of fibro-carcinogenesis, possibly now driven by genetic and epigenetic alteration, has already begun before HCV clearance (manuscript in preparation). In this setting, a backswitch of TGF-β/Smad sig-

naling from fibro-carcinogenic to cytostatic cannot be achieved due to passage across a 'point of no return' [32–34].

The lab of Mikulits has also intensely investigated the transformation of hepatocytes into cancer cells with a focus on the TGF-β signaling pathway. Many of their studies were performed with hepatocytes isolated from p19(ARF)–/– mice. Loss of p19(ARF) lowers the growth-suppressive functions of p53 and bypasses cellular senescence without loss of genetic stability. Thus, it was possible to generate immortalized murine hepatocytes, which display a high degree of differentiation and display arrest in the G1 phase under exposure to TGF-β. These hepatocytes maintain epithelial polarization upon expression of oncogenic Ha-Ras. However, Ras-transformed hepatocytes rapidly convert to a spindle-shaped, fibroblastoid morphology upon treatment with TGF-β, which no longer inhibits proliferation [35]. In further studies, the lab of Mikulits showed that loss of E-cadherin, increased LEF/TCF-β-catenin, as well as PDGF signaling and c-Fos cooperate with autocrine TGF-β signaling to maintain an undifferentiated mesenchymal 'hepatocyte' phenotype [36–40]. In addition, the role of TGF-β in cell-cell communication between HCC cells and intra-/peritumorally accumulated, activated HSC-derived, stroma myofibroblasts was investigated. By employing cellular transplantation, interaction of neoplastic hepatocytes with the tumor microenvironment containing activated HSC or myofibroblasts derived thereof induces increased nuclear localization of Smad2/3 and β-catenin, thereby facilitating malignant progression. Interference with TGF-β signaling by Smad7 expression in hepatocytes diminished nuclear β-catenin, epithelial dedifferentiation, and tumor progression, indicating a crosstalk between TGF-β and β-catenin signaling. The authors conclude that HSC-derived myofibroblasts directly govern hepatocarcinogenesis in a TGF-β-dependent fashion by inducing autocrine TGF-β signaling and nuclear β-catenin accumulation in neoplastic hepatocytes. Similar results monitoring the invasive behavior of malignant cells were obtained in collagen gel-based three-dimensional micro-organoid HCC spheroids; it was significantly diminished after inhibition of TGF-β or PDGF signaling. The authors conclude that the TGF-β/PDGF/β-catenin axis in malignant hepatocytes is triggered from hepatic tumor-stroma crosstalk and is crucial for both tumor growth and cancer progression [41–43].

Giannelli et al. [44] showed that TGF-β stimulates α3-integrin expression in malignant hepatocytes, which triggers their transformation into a motile and invasive phenotype. In HCC patients, TGF-β1 serum concentrations and α3-integrin expression are strongly correlated [44]. They further showed that in HCC of human patients, Ln-5, Snail, and Slug are upregulated, E-cadherin is downregulated, and β-catenin is translocated into the nuclei. In vitro, Ln-5 mediates partial EMT, upregulation of Snail and Slug, and downregulation of E-cadherin in HCC 'invasive' cells which, however, do not scatter. In the presence of both Ln-5 and TGF-β, the EMT process is completed, β-catenin is translocated into the nuclei, and cells scatter and become invasive. The process can be reversed by anti-α3 integrin blocking antibody [45].

Human HCC cell lines were treated with an ALK-5 inhibitor (LY2109761), which selectively blocks TGF-β-induced pSmad2 and dephosphorylates autocrine pSmad2 at concentrations ranging from 0.001 to 0.1 μM. The drug upregulates E-cadherin mRNA and protein levels, inhibits migration on fibronectin, laminin-5, and vitronectin, and abrogates invasion through Matrigel. Furthermore, nonmetastatic HCC tissues from 7 patients were cultured with TGF-β in the presence or absence of LY2109761. E-cadherin expression was reduced by TGF-β and was significantly increased upon LY2109761 treatment, as measured by quantitative real-time PCR on microdissected tissues and by immunohistochemistry on serial sections. In 72 patients, E-cadherin tissue expression was more weakly expressed in metastatic HCC than in nonmetastatic HCC [46].

In another study, the Giannelli lab showed that HCC cells invade blood vessels via α5β1-integrin, which, however, is equally expressed in invasive and noninvasive cells and requires phosphorylation of the intracytoplasmic tail at threonine 788–789. This is achieved by TGF-β via Smad2 and Smad3, thereby modulating noninvasive HCC cells to behave like invasive cells. An ALK-5 inhibitor efficiently interferes with α5β1-integrin phosphorylation and blocks invasion of HCC cells. This mechanism was confirmed in human HCC patients with microvascular invasion that displayed pα5β1-integrin, TGF-β1, pSmad2, and E-cadherin, indicating that TGF-β1 promotes vascular invasion by activating α5β1-integrin [47].

Further, Giannelli and coworkers showed that treatment of HCC with the ALK-5 inhibitor LY2109761 inhibits molecular pathways involved in neo-angiogenesis and tumor growth. Interestingly, this anti-angiogenic effect is more effective than that of bevacizumab, which specifically targets VEGF. Mechanistically, LY210976 disrupted the paracrine crosstalk between HCC and endothelial cells that involved Smad2/3-mediated signaling and af-

fected the secretion of VEGF, thus inhibiting blood vessel formation [48].

Finally, the Giannelli group took into account tumor-stroma interactions and showed that HCC invasive cells produce high levels of CTGF and generate tumors with a high stromal component in a xenograft model. LY2109761 treatment inhibited the synthesis and release of CTGF from malignant hepatocytes and reduced the stromal component of the tumors. Furthermore, the decrease in CTGF production diminished tumor growth, intravasation, and metastatic dissemination of HCC cells by inhibiting cancer-associated fibroblast proliferation. Moreover, TGF-β treatment was able to enhance CTGF production in noninvasive HCC cells, which subsequently form tumors with a high stromal content, acquired intravasation, and metastatic spread, all of which could be blunted by treatment with LY2109761 [49].

Taken together, preclinical results from the Giannelli lab indicate that the TGF-β/ALK-5 inhibitor LY2109761 targets the crosstalk between HCC, stroma myofibroblast, and vascular endothelial cells with a strong impact against tumor progression and malignancy, thus providing a rationale for future clinical trials.

A very impressive clinical study was performed in the Thorgeirsson lab by Coulouarn et al. [50]. Taking into account the clinical heterogeneity of HCC and the highly variable clinical course of HCC patients, they assumed that biological subgrouping could provide a better molecular classification of HCC useful for prognostic predictions and to select treatment options. Based on the knowledge of TGF-β as a potential provider of both tumor-suppressive (growth inhibition, apoptosis) and oncogenic (EMT, invasiveness, etc.) properties, they selected TGF-β gene expression signatures in such an approach to refine the classification and prognostic predictions for HCC patients. Therefore, Coulouarn et al. [50] first established a temporal TGF-β gene expression signature in mouse hepatocytes. Applying then a comparative functional genomics approach comprising 139 HCC patients, they were able to successfully discriminate distinct subgroups of HCC. The TGF-β-positive cluster included two novel homogeneous groups of HCC associated with early and late TGF-β signatures. Kaplan-Meier plots and log-rank statistics indicated that the patients with a late TGF-β signature showed significantly shortened mean survival times (16.2 ± 5.3 months) compared to the patients with an early (60.7 ± 16.1 months) TGF-β signature.

In a similar approach, the authors had previously subclassified the same HCC patients as having either bad or good prognoses with regard to survival, hepatocytes versus hepatoblasts, and HGF/c-Met positive versus negative gene expression signatures. With the addition of early and late TGF-β signatures into these profiles, hepatoblast-HGF/c-Met positive/late TGF-β signatures could be correlated with the worst patient outcome.

Also, tumors expressing late TGF-β responsive genes displayed an invasive phenotype and increased tumor recurrence. Furthermore, a late TGF-β signature was able to accurately predict liver metastasis and discriminated HCC cell lines by degree of invasiveness.

In the group of Dooley, two different culture setups for primary mouse hepatocytes are in use, i.e. a collagen sandwich or a collagen monolayer, as previously reported [51]. In the collagen sandwich, hepatocytes maintain polarity and are able to form bile canaliculi for up to 2 weeks, therewith conserving hepatocyte physiological functions to a relatively large extent. In collagen monolayer cultures, hepatocytes lose their orientation and polarity. In this setting, especially mouse hepatocytes rapidly display a stress response, either inducing a survival program with gain of plasticity and transdifferentiation into cells with myofibroblast features or alternatively entering apoptosis. The collagen monolayer induces focal adhesion kinase (FAK) via Src, which leads to activation of AKT and extracellular signal-regulated kinase (ERK) 1/2 pathways. AKT causes resistance to TGF-β-induced apoptosis by antagonizing p38, whereas ERK1/2 signaling opens the route to EMT.

In contrast to the collagen monolayer, in the collagen sandwich FAK activation does not occur, keeping the hepatocytes in a state where they remain sensitive to TGF-β-induced apoptosis and do not undergo EMT. In this culture system, inhibition of p38 as well as overexpression of constitutively active AKT causes apoptosis resistance, whereas constitutively active Ras induces EMT. Interestingly, matrix-induced EMT can be reversed by replating cells from the collagen monolayer into the collagen sandwich system, indicating that hepatocyte dedifferentiation in vitro is an active process driven by FAK-mediated AKT and ERK1/2 signaling [51].

In addition, hepatocytes cultured on a collagen monolayer display a high basal frequency of proliferation. In order to identify the mechanisms initiating hepatocyte 'plasticity' and priming for proliferation in further detail, gene expression patterns were investigated and genes related to MAPK signaling and the cell cycle were found to be upregulated, with the latter having an overrepresentation of transcription factor binding sites for ETF (TEA domain family member 2), E2F1 (E2F transcription factor 1), and SP-1 (Sp1 transcription factor), all depending

on MAPK signaling. ERK1/2 phosphorylation was identified as an early event, accompanied by proliferative activity, as measured by bromodeoxyuridine labeling. The MEK inhibitor PD98059 blunted these effects, indicating MAPK signaling as a major trigger for the hepatocyte proliferative response. Furthermore, hepatocytes with pERK1/2 staining and nuclear SP-1 and E2F1 expression were found in liver tissue of mice challenged with CCl4, confirming that hepatocytes cultured as a collagen monolayer are primed for proliferation through activation of MAPK signaling that induces a proliferative expression signature via transcription factors ETF, E2F1, and SP-1 [52].

Human and rat hepatocytes are more stable when cultured in the collagen monolayer system and keep polarity for about a week, whereas mouse hepatocytes display this stress response already at day 2 in culture.

Interestingly, stressed hepatocytes resist cytostatic TGF-β signaling without decreasing responsiveness, e.g. measured as TGF-β-mediated Smad activation. Instead, the TGF-β pathway further facilitates and enhances the aforementioned transdifferentiation process. That is achieved by a caveolin-1-dependent non-Smad signaling-mediated increase in pAKT [53] and a Smad-dependent gene expression profile that reflects the aforementioned late TGF-β signature [50], including, among others, upregulation of vimentin, Snail, Slug, VEGF, CTGF, TGF-β, TIMP-1, PDGF, Col1A1/2, PAI-1, TSP-1, N-cadherin, and phalloidin and downregulation of E-cadherin, ZO-1, and claudin-1. All of these Smad-mediated effects can be blunted by ectopic or transgenic expression of Smad7 in vitro and in vivo [54].

This further supports the role of TGF-β as: (i) an inducer of EMT and (ii) a producer of secretory proteins for tumor cell/stroma communication in malignant hepatocytes. Especially the fact that these tumorigenic TGF-β actions may be redirected to cytostasis just by interfering with AKT signaling might be of high pharmaceutical interest.

In summary, HCC can be characterized as a cancer type in which activation of the TGF-β signaling pathway is not disrupted and plays a major role in tumor progression to malignancy. The tumor-promoting action of TGF-β is achieved by selective inhibition of the tumor suppressor arm early in malignant transformation that involves several molecular mechanisms. Importantly, in this scenario the TGF-β signal is redirected towards tumor progression and malignancy via expression signatures mediating hepatocyte plasticity and EMT and encoding secreted proteins that act on the tumor environment. Interestingly, in early transformed stages tumorigenic TGF-β signaling can be reverted to cytostatic by interfering with survival signaling, which may have an important impact on the development of therapeutic approaches aiming to interfere with liver carcinogenesis. This is schematically summarized in figure 1.

Since molecular targeting of the TGF-β pathway is experimentally well established and is currently being tested in a clinical study to treat patients with progressed HCC, the above data additionally advise that a combined approach comprising interference with survival signaling plus activation of TGF-β signaling may induce HCC cell death and may thus be efficient in the early stages of HCC development.

Acknowledgements

Our research on chronic liver disease and HCC is supported by the German Research Foundation programs 'SFB TRR77 Liver Cancer' and 'Do373/8-1', and by Federal Ministry of Education and Research grants 'The Virtual Liver' and 'Cell Therapy in Liver Regeneration' (S.D. and N.M.B.) and the Europäischer Sozialfonds in Baden-Württemberg. We are grateful to Drs. Lopa Mishra (MD Anderson Cancer Center, Houston, Tex., USA), Keigo Machida (USC, Los Angeles, Calif., USA), Wolfgang Mikulits (Medical University, Vienna, Austria), and Gianluigi Giannelli (University of Bari Medical School) for providing detailed information about their work. The current review is not exhaustive and the references are chosen as illustrations. We apologize to the authors whose work is not cited here.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

References

1 Moustakas A, Heldin CH: Non-Smad TGF-beta signals. J Cell Sci 2005;118:3573–3584.
2 Nakashima K, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Kawabata M, Miyazono K, Taga T: Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. Science 1999;284:479–482.
3 Matsuzaki K: Smad phosphoisoform signals in acute and chronic liver injury: similarities and differences between epithelial and mesenchymal cells. Cell Tissue Res 2012;347:225–243.
4 Park SH: Fine tuning and cross-talking of tgf-beta signal by inhibitory smads. J Biochem Mol Biol 2005;38:9–16.
5 Taylor IW, Wrana JL: Snapshot: The TGF-beta pathway interactome. Cell 2008;133:378.e1.

6 Siegel PM, Massague J: Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer 2003;3:807–821.
7 Breitkopf K, Godoy P, Ciuclan L, Singer MV, Dooley S: TGF-beta/Smad signaling in the injured liver. Z Gastroenterol 2006;44:57–66.
8 Karkampouna S, Ten Dijke P, Dooley S, Kruithof-de Julio F: TGFβ signaling pathway implication in liver regeneration. Curr Pharm Des 2012, E-pub ahead of print.
9 Gressner AM, Weiskirchen R: Modern pathogenetic concepts of liver fibrosis suggest stellate cells and TGF-beta as major players and therapeutic targets. J Cell Mol Med 2006;10:76–99.
10 Braun L, Mead JE, Panzica M, Mikumo R, Bell GI, Fausto N: Transforming growth factor-beta mRNA increases during liver regeneration: a possible paracrine mechanism of growth regulation. Proc Natl Acad Sci USA 1988;85:1539–1543.
11 Michalopoulos GK, DeFrances MC: Liver regeneration. Science 1997;276:60–66.
12 Padua D, Massague J: Roles of TGFbeta in metastasis. Cell Res 2009;19:89–102.
13 Farazi PA, DePinho RA: Hepatocellular carcinoma pathogenesis: from genes to environment. Nat Rev Cancer 2006;6:674–687.
14 Baek HJ, Pishvaian MJ, Tang Y, Kim TH, Yang S, Zouhairi ME, Mendelson J, Shetty K, Kallakury B, Berry DL, Shin KH, Mishra B, Reddy EP, Kim SS, Mishra L: Transforming growth factor-beta adaptor, beta2-spectrin, modulates cyclin dependent kinase 4 to reduce development of hepatocellular cancer. Hepatology 2011;53:1676–1684.
15 Thenappan A, Shukla V, Abdul Khalek FJ, Li Y, Shetty K, Liu P, Li L, Johnson RL, Johnson L, Mishra L: Loss of transforming growth factor beta adaptor protein beta-2 spectrin leads to delayed liver regeneration in mice. Hepatology 2011;53:1641–1650.
16 Thenappan A, Li Y, Kitisin K, Rashid A, Shetty K, Johnson L, Mishra L: Role of transforming growth factor beta signaling and expansion of progenitor cells in regenerating liver. Hepatology 2010;51:1373–1382.
17 Mishra L, Banker T, Murray J, Byers S, Thenappan A, He AR, Shetty K, Johnson L, Reddy EP: Liver stem cells and hepatocellular carcinoma. Hepatology 2009;49:318–329.
18 Baek HJ, Lim SC, Kitisin K, Jogunoori W, Tang Y, Marshall MB, Mishra B, Kim TH, Cho KH, Kim SS, Mishra L: Hepatocellular cancer arises from loss of transforming growth factor beta signaling adaptor protein embryonic liver fodrin through abnormal angiogenesis. Hepatology 2008;48:1128–1137.
19 Lin L, Amin R, Gallicano GI, Glasgow E, Jogunoori W, Jessup JM, Zasloff M, Marshall JL, Shetty K, Johnson L, Mishra L, He AR: The STAT3 inhibitor NSC 74859 is effective in hepatocellular cancers with disrupted TGF-beta signaling. Oncogene 2009;28:961–972.
20 Kitisin K, Ganesan N, Tang Y, Jogunoori W, Volpe EA, Kim SS, Katuri V, Kallakury B, Pishvaian M, Albanese C, Mendelson J, Zasloff M, Rashid A, Fishbein T, Evans SR, Sidawy A, Reddy EP, Mishra B, Johnson LB, Shetty K, Mishra L: Disruption of transforming growth factor-beta signaling through beta-spectrin eELF leads to hepatocellular cancer through cyclin D1 activation. Oncogene 2007;26:7103–7110.
21 Katuri V, Tang Y, Li C, Jogunoori W, Deng CX, Rashid A, Sidawy AN, Evans S, Reddy EP, Mishra B, Mishra L: Critical interactions between TGF-beta signaling/ELF, and E-cadherin/beta-catenin mediated tumor suppression. Oncogene 2006;25:1871–1886.
22 Saha T, Vardhini D, Tang Y, Katuri V, Jogunoori W, Volpe EA, Haines D, Sidawy A, Zhou X, Gallicano I, Schlegel R, Mishra B, Mishra L: RING finger-dependent ubiquitination by PRAJA is dependent on TGF-beta and potentially defines the functional status of the tumor suppressor ELF. Oncogene 2006;25:693–705.
23 Tang Y, Kitisin K, Jogunoori W, Li C, Deng CX, Mueller SC, Ressom HW, Rashid A, He AR, Mendelson JS, Jessup JM, Shetty K, Zasloff M, Mishra B, Reddy EP, Johnson L, Mishra L: Progenitor/stem cells give rise to liver cancer due to aberrant TGF-beta and IL-6 signaling. Proc Natl Acad Sci USA 2008;105:2445–2450.
24 Tang Y, Katuri V, Dillner A, Mishra B, Deng CX, Mishra L: Disruption of transforming growth factor-beta signaling in elf beta-spectrin-deficient mice. Science 2003;299:574–577.
25 Amin R, Mishra L: Liver stem cells and tgf-beta in hepatic carcinogenesis. Gastrointest Cancer Res 2008;2:S27–30.
26 Machida K, Tsukamoto H, Mkrtchyan H, Duan L, Dynnyk A, Liu HM, Asahina K, Govindarajan S, Ray R, Ou JH, Seki E, Deshaies R, Miyake K, Lai MM: Toll-like receptor 4 mediates synergism between alcohol and HCV in hepatic oncogenesis involving stem cell marker Nanog. Proc Natl Acad Sci USA 2009;106:1548–1553.
27 Ferrigno O, Lallemand F, Verrecchia F, L'Hoste S, Camonis J, Atfi A, Mauviel A: Yes-associated protein (YAP65) interacts with Smad7 and potentiates its inhibitory activity against TGF-beta/Smad signaling. Oncogene 2002;21:4879–4884.
28 Machida K, Chen CL, Liu JC, Kashiwabara C, Feldman D, French SW, Sher L, Hyeongnam JJ, Tsukamoto H: Cancer stem cells generated by alcohol, diabetes, and hepatitis C virus. J Gastroenterol Hepatol 2012;27(suppl 2):19–22.
29 Matsuzaki K: Smad phosphoisoform signaling specificity: the right place at the right time. Carcinogenesis 2011;32:1578–1588.
30 Nagata H, Hatano E, Tada M, Murata M, Kitamura K, Asechi H, Narita M, Yanagida A, Tamaki N, Yagi S, Ikai I, Matsuzaki K, Uemoto S: Inhibition of c-Jun NH2-terminal kinase switches Smad3 signaling from oncogenesis to tumor-suppression in rat hepatocellular carcinoma. Hepatology 2009;49:1944–1953.
31 Murata M, Matsuzaki K, Yoshida K, Sekimoto G, Tahashi Y, Mori S, Uemura Y, Sakaida N, Fujisawa J, Seki T, Kobayashi K, Yokote K, Koike K, Okazaki K: 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 2009;49:1203–1217.
32 Furukawa F, Matsuzaki K, Mori S, Tahashi Y, Yoshida K, Sugano Y, Yamagata H, Matsushita M, Seki T, Inagaki Y, Nishizawa M, Fujisawa J, Inoue K: p38 MAPK mediates fibrogenic signal through Smad3 phosphorylation in rat myofibroblasts. Hepatology 2003;38:879–889.
33 Matsuzaki K, Murata M, Yoshida K, Sekimoto G, Uemura Y, Sakaida N, Kaibori M, Kamiyama Y, Nishizawa M, Fujisawa J, Okazaki K, Seki T: Chronic inflammation associated with hepatitis C virus infection perturbs hepatic transforming growth factor beta signaling, promoting cirrhosis and hepatocellular carcinoma. Hepatology 2007;46:48–57.
34 Yoshida K, Matsuzaki K, Mori S, Tahashi Y, Yamagata H, Furukawa F, Seki T, Nishizawa M, Fujisawa J, Okazaki K: 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 2005;166:1029–1039.
35 Gotzmann J, Huber H, Thallinger C, Wolschek M, Jansen B, Schulte-Hermann R, Beug H, Mikulits W: Hepatocytes convert to a fibroblastoid phenotype through the cooperation of TGF-beta1 and Ha-Ras: steps towards invasiveness. J Cell Sci 2002;115:1189–1202.
36 Mikula M, Gotzmann J, Fischer AN, Wolschek MF, Thallinger C, Schulte-Hermann R, Beug H, Mikulits W: The proto-oncoprotein c-Fos negatively regulates hepatocellular tumorigenesis. Oncogene 2003;22:6725–6738.
37 Eger A, Stockinger A, Park J, Langkopf E, Mikula M, Gotzmann J, Mikulits W, Beug H, Foisner R: Beta-catenin and tgfbeta signalling cooperate to maintain a mesenchymal phenotype after foser-induced epithelial to mesenchymal transition. Oncogene 2004;23:2672–2680.
38 Gotzmann J, Fischer AN, Zojer M, Mikula M, Proell V, Huber H, Jechlinger M, Waerner T, Weith A, Beug H, Mikulits W: A crucial function of PDGF in TGF-beta-mediated cancer progression of hepatocytes. Oncogene 2006;25:3170–3185.

39 Fischer AN, Fuchs E, Mikula M, Huber H, Beug H, Mikulits W: PDGF essentially links TGF-beta signaling to nuclear beta-catenin accumulation in hepatocellular carcinoma progression. Oncogene 2007;26:3395–3405.
40 Lahsnig C, Mikula M, Petz M, Zulehner G, Schneller D, van Zijl F, Huber H, Csiszar A, Beug H, Mikulits W: ILEI requires oncogenic Ras for the epithelial to mesenchymal transition of hepatocytes and liver carcinoma progression. Oncogene 2009;28:638–650.
41 Mikula M, Proell V, Fischer AN, Mikulits W: Activated hepatic stellate cells induce tumor progression of neoplastic hepatocytes in a TGF-beta dependent fashion. J Cell Physiol 2006;209:560–567.
42 van Zijl F, Mair M, Csiszar A, Schneller D, Zulehner G, Huber H, Eferl R, Beug H, Dolznig H, Mikulits W: Hepatic tumor-stroma crosstalk guides epithelial to mesenchymal transition at the tumor edge. Oncogene 2009;28:4022–4033.
43 Zulehner G, Mikula M, Schneller D, van Zijl F, Huber H, Sieghart W, Grasl-Kraupp B, Waldhor T, Peck-Radosavljevic M, Beug H, Mikulits W: Nuclear beta-catenin induces an early liver progenitor phenotype in hepatocellular carcinoma and promotes tumor recurrence. Am J Pathol 2010;176:472–481.
44 Giannelli G, Fransvea E, Marinosci F, Bergamini C, Colucci S, Schiraldi O, Antonaci S: Transforming growth factor-beta1 triggers hepatocellular carcinoma invasiveness via alpha3beta1 integrin. Am J Pathol 2002;161:183–193.
45 Giannelli G BC, Fransvea E, Sgarra C, Antonaci S: Laminin-5 with transforming growth factor-beta1 induces epithelial to mesenchymal transition in hepatocellular carcinoma. Gastroenterology 2005;129:1375–1383.
46 Fransvea E, Angelotti U, Antonaci S, Giannelli G: Blocking transforming growth factor-beta up-regulates E-cadherin and reduces migration and invasion of hepatocellular carcinoma cells. Hepatology 2008;47:1557–1566.
47 Fransvea E, Mazzocca A, Antonaci S, Giannelli G: Targeting transforming growth factor (TGF)-betaRI inhibits activation of beta1 integrin and blocks vascular invasion in hepatocellular carcinoma. Hepatology 2009;49:839–850.
48 Mazzocca A, Fransvea E, Lavezzari G, Antonaci S, Giannelli G: Inhibition of transforming growth factor beta receptor I kinase blocks hepatocellular carcinoma growth through neo-angiogenesis regulation. Hepatology 2009;50:1140–1151.
49 Mazzocca A, Fransvea E, Dituri F, Lupo L, Antonaci S, Giannelli G: Down-regulation of connective tissue growth factor by inhibition of transforming growth factor beta blocks the tumor-stroma cross-talk and tumor progression in hepatocellular carcinoma. Hepatology 2010;51:523–534.
50 Coulouarn C, Factor VM, Thorgeirsson SS: Transforming growth factor-beta gene expression signature in mouse hepatocytes predicts clinical outcome in human cancer. Hepatology 2008;47:2059–2067.
51 Godoy P, Hengstler JG, Ilkavets I, Meyer C, Bachmann A, Muller A, Tuschl G, Mueller SO, Dooley S: Extracellular matrix modulates sensitivity of hepatocytes to fibroblastoid dedifferentiation and transforming growth factor beta-induced apoptosis. Hepatology 2009;49:2031–2043.
52 Zellmer S, Schmidt-Heck W, Godoy P, Weng H, Meyer C, Lehmann T, Sparna T, Schormann W, Hammad S, Kreutz C, Timmer J, von Weizsacker F, Thurmann PA, Merfort I, Guthke R, Dooley S, Hengstler JG, Gebhardt R: Transcription factors ETF, E2F, and SP-1 are involved in cytokine-independent proliferation of murine hepatocytes. Hepatology 2010;52:2127–2136.
53 Meyer C, Godoy P, Bachmann A, Liu Y, Barzan D, Ilkavets I, Maier P, Herskind C, Hengstler JG, Dooley S: Distinct role of endocytosis for Smad and non-Smad TGF-beta signalling regulation in hepatocytes. J Hepatol 2011;55:369–378.
54 Dooley S, Hamzavi J, Ciuclan L, Godoy P, Ilkavets I, Ehnert S, Ueberham E, Gebhardt R, Kanzler S, Geier A, Breitkopf K, Weng H, Mertens PR: Hepatocyte-specific Smad7 expression attenuates TGF-beta-mediated fibrogenesis and protects against liver damage. Gastroenterology 2008;135:642–659.