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Tumor-Suppressive and Tumor-Promoting Role of Tgf-β in Hepatocellular Carcinoma

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Abstract

Tgf-β is a pleiotropic cytokine with diverse functions on hepatic cells. The well-known function of Tgf-β in pathogenesis of liver disease is to stimulate liver fibrosis that often precedes the onset of liver cancer. While Tgf-β-mediated fibrosis seems to make liver more prone to the development of liver cancer, Tgf-β suppresses initial malignant transformation of hepatic cells thru regulation of proliferation and apoptosis. On the other hand, Tgf-β has shown to act as an inducer of tumor development thru enhancement of metastatic process. Additionally, it has been shown that Tgf-β signaling in hepatocytes promotes hepatocarcinogenesis caused by certain genetic conditions. This review highlights observations that have improved an understanding of how Tgf-β contributes to the development of hepatocellular carcinoma.

Keywords: Tgf-β, fibrosis, chronic liver disease, Hepatocellular Carcinoma

1. Introduction

Hepatocellular carcinoma (HCC) is among the most common cancer that causes increasing morbidity and mortality every year (Ferlay et al., 2010; Nordenstedt, White, & El-Serag, 2010). HCC frequently develops in patient with clinical history of chronic liver injury (Bissell, 2001) . There are several major risk factors for HCC. Hepatitis B (HBV) and Hepatitis C Virus (HCV) infection represents the major risk factor for HCC worldwide whereas alcoholic-induced liver injury, nonalcoholic steatohepatitis (NASH), autoimmune disorders are also considered important contributors (Ferlay et al., 2010; Nordenstedt et al., 2010).Other risk factors include genetically determined disorders, such as cystic fibrosis (O'Donnell, Ryan, Hayes, Fennelly, & Gibney, 2009), hereditary hemochromatosis (Kowdley, 2004) and Wilson's disease (Kumagi et al., 2004).

When liver is subjected to the pathological conditions, compromised hepatocytes trigger reparative response that consists of fibrosis and inflammation. Chronic injury in diseased livers leads to increased number of reactive cells such as fibrogenic fibroblasts and inflammatory cells. These reactive cells produce various types of potent tropic factor and cytokine that contributes to a malignant transformation of hepatocyte into HCC. Among factors known to be involved in HCC development, Transforming growth factor (Tgf-β) has shown to play diverse functions in liver carcinogenesis. The role of Tgf-β appears to favor HCC development in pre-cancerous stage since it promote the accumulation of myofibroblasts and inflammation that are source of inducing factors for malignant transformation. On the other hand, Tgf-β has shown to regulate proliferation and apoptosis of pre-malignant hepatocyte and, therefore, inhibit hepatocarcinogenesis. Interestingly, it has been shown that Tgf-β accelerates HCC development caused by specific gene mutations. This review focuses on findings that shed light on mechanism in which Tgf-β influences tumorigenesis of HCC.

2. Overview of Tgf-β Signaling

Tgf-β belongs to the superfamily of cytokines that include Bone Morphogenetic Protein (BMP), growth differentiation factor (GDF), anti-mullerian hormone (AMH), myostain, activin and inhibin (Piek, Heldin, & Ten Dijke, 1999). Three highly homologous Tgf-β isoforms such as Tgf-β1, Tgf-β2, Tgf-β3 have been identified in mammalian cells. Although all three isoforms transduce similar signaling transduction pathway, They were shown to have quite different biological response (Massague & Chen, 2000). Tgf-βs initiate the signaling transduction via two types of cell surface receptors type II and type I that signal thru cytoplasmic serine/theronine kinase domain (Attisano & Wrana, 2002). Upon ligand binding, type II receptors (TβRII) recruits type I receptor (TβRI, activin

receptor-like kinases, Alks). Interaction between TβRII and TβRI allows the kinase domain of TβRII to phosphorylate Alks in a glycine- and serine-rich juxtamembrane region called GS domain which is an essential step that initiate downstream signaling cascades. Phosphorylated Alks then activate the receptor-activated Smads (R-Smads) that allows them to bind to the common Smads (Co-Smads). R-Smad/Co-Smad complex subsequently translocates into nucleus where it acts as transcription factors and regulate expression of many target genes (Heldin, Miyazono, & ten Dijke, 1997; Massague & Chen, 2000).

Mammalian cells express five type II receptors (BMPRII, ActRII, ActRIIB, TβRII and AMHR), seven type I receptors (Alk1-Alk7), five R-Smads (Smad1, Smad2, Smad3, Smad5 and Smad8), one Co-Smad (Smad4), and two inhibitory (I)-Smads (Smad6 and Smad7). Alk1, Alk2, Alk3 and Alk6 phosphorylate Smad1, Smad5 and Smad8 whereas Alk4, Alk5 and Alk7 activate Smad2 and Smad3 (Derynck & Zhang, 2003; Piek et al., 1999; ten Dijke & Hill, 2004). While Tgf-β signals TβRII-Alk5-Smad2/3 pathway in most cells, previous studies have shown that Tgf-β initiates Alk1-Smad1/5 signaling pathway in addition to activation of Alk5-Smad2/3 cascade in certain cell type such as endothelial cells and Hepatic Stellate Cells (Goumans et al., 2003; Wiercinska et al., 2006).

Several line of evidence has shown that the transduction of Tgf-β signaling leads to increased expression of I-Smad that enables inhibition of Tgf-β-mediated response (Chen, Huang, Morinelli, Trojanowska, & Paul, 2002; Nakao et al., 1997; von Gersdorff et al., 2000). There are many way that Tgf-β signaling can be downregulated by I-Smad. Smad 6/7 is able to block recruitment of Alks to Tgf-β-TβRII complex and suppress phosphorylation of Smad2/3(Y. Shi & Massague, 2003; Zhao, Shi, Chen, & Warburton, 2000). Smad7 also promote degradation of type II-type I receptor complexes by recruiting E3-ubiquitin ligase (Ebisawa et al., 2001). Additionally, Smad7 was shown to mediate dephosphorylation and inactivation of Alk5 thru the recruitment of GADD34 complex and the catalytic subunit of protein phosphatase 1 (W. Shi et al., 2004).

3. Tgf-β in Pre-Cancerous Stage of HCC

The role of Tgf-βs in organ fibrosis and collagen synthesis was identified more than 20 years ago (Czaja et al., 1989). The increased expression of Tgf-β family members are often found in regions of extracellular matrix (ECM) deposition in many fibrotic diseases (Branton & Kopp, 1999). Tgf-βs have been implicated as an important contributor of fibrosis in several liver diseases (Gressner, Weiskirchen, Breitkopf, & Dooley, 2002). In patients with chronic liver diseases, an increased in Tgf-βs expression was observed. For example, high level of Tgf-βs is found during hepatic fibrosis in patients with hepatitis B, Hepatitis C (Kirmaz et al., 2004). An increased level of Tgf-βs was also detected in peripheral blood mononuclear cells of alcoholic cirrhosis patients (W. X. Chen et al., 2002). As their important functions in fibrotic liver diseases became more apparent, the level of Tgf-β is considered as potential indicator for prognosis (Kirmaz et al., 2004).

The increased level of Tgf-βs is thought to be derived from damaged hepatic epithelium as well as from stromal cells including hepatic Stellate Cells (HSC), portal fibroblasts and inflammatory cells. In fibrotic liver from patient with cystic fibrosis liver disease (CFLD), cholangiocytes was shown to be the main source of Tgf-βs (Lewindon et al., 2002). A study of fibrotic liver from patients and animal model of cholestatic liver diseases indicated that mesenchymal cells and inflammatory cells predominantly expressed Tgf-βs (Milani, Herbst, Schuppan, Stein, & Surrenti, 1991). Additionally, High level of Tgf-βs was reported in perisinusoidal cells of liver obtained from rodents exposed to hepatotoxic carbon tetrachloride (Czaja et al., 1989). The observations imply that a selective expression of Tgf-β ligands might play a critical role in liver fibrosis and regeneration

Tgf-β is best known for regulation of HSC in fibrotic liver disease. This notion is supported by various types of evidence from *in vivo* and *in vitro* studies. Following hepatocyte injury, Tgf-β up-regulates expression of its receptors and plasminogen activator inhibitor type 1 (PAI-1) in HSC (Knittel et al., 1999; Rieder, Armbrust, Meyer zum Buschenfelde, & Ramadori, 1993). While Tgf-β was shown to inhibit quiescent HSC proliferation induced by platelet-derived growth factor (PDGF), Tgf-β shows stimulatory effect on expansion of transdifferentiation of myofibroblasts (Dooley, Delvoux, Lahme, Mangasser-Stephan, & Gressner, 2000). HSCs also respond to Tgf-β by up-regulating type I collagen and Tissue inhibitor of Metalloproteinase-1 and -2 (TIMP-1 and -2) (Herbst et al., 1997). These HSC respond to Tgf-β is mediated by Smad3 signaling pathway. Smad3 was shown to mediate Alk5-mediated fibrogenic response of HSC thru the induction of collagen synthesis (Furukawa et al., 2003; Inagaki, Mamura, et al., 2001; Inagaki, Nemoto, et al., 2001; Schnabl et al., 2001; Seyhan et al., 2006). It has also been shown that Smad1 is involved in Tgf-β-induced fibrogenic response thru stimulation of inhibitor of DNA binding 1 (Id1). Study has shown that Id1 is necessary for Tgf-β-induced response and its overexpression is sufficient induce HSC activation (Wiercinska et al., 2006).

Moreover, Tgf-β-mediated HSC response is under a functional negative feedback regulation by Smad7.In activated HSCs obtained from fibrotic rodent livers and from *in vitro* stimulations, a strong R-Smad activation is

observed whereas Smad7 expression is diminished (Dooley et al., 2000; Dooley, Delvoux, et al., 2001; Dooley, Streckert, Delvoux, & Gressner, 2001; Liu et al., 2003; Stopa, Anhuf, et al., 2000; Stopa, Benes, Ansorge, Gressner, & Dooley, 2000; Tahashi et al., 2002). These finding suggest that suppression of Smad7 induction might contribute to fully stimulated Tgf-β signaling in HSC during the progression of hepatic fibrosis.

In addition to regulating fibrogenesis, Tgf-β has been found to modulate inflammatory response. Tgf-β have been implicated in systemic inflammation since increased Tgf-β level was observed in sepsis (Marie, Cavaillon, & Losser, 1996) and post-traumatic shock (Laun et al., 2003; Varedi, Jeschke, Englander, Herndon, & Barrow, 2001). Tgf-β has been found to promote inflammatory cytokines Tumor necrosis factor-α (TNF-α)(Chantry, Turner, Abney, & Feldmann, 1989) and Interleukin-6 (IL-6) (Turner, Chantry, & Feldmann, 1990). Transgenic mice over-expressing Tgf-β were more susceptible to endotoxemia (Vodovotz et al., 1998). The phenotype was associated with increased LPS-induced inflammatory cytokine secretion by liver cells (Garcia-Lazaro et al., 2005). It has also been reported that ectopic expression of Tgf-β increases the immune infiltration on the liver in mouse model of alcoholic liver disease (Preisegger et al., 1999). This reveals the pro-inflammatory effect of Tgf-β in hepatocytes during pathogenesis of liver disease. Alternatively, it is also possible that Tgf-β exhibits its effect thru the enhancement of Th17 response since it has been shown that Tgf-β can act as differentiation factor for Th17 cell (Mangan et al., 2006; Veldhoen, Hocking, Atkins, Locksley, & Stockinger, 2006) that plays an important role in various types of inflammatory diseases (Bettelli, Oukka, & Kuchroo, 2007). Since chronic inflammation and fibrosis are well-known to be risk factors for tumorigenesis (Grivennikov & Karin, 2010), an increased Tgf-β activity in affected liver seems to favor the onset of HCC.

4. Tgf-β as Tumor Suppressor

Results of cell culture experiments have revealed an importance of Tgf-β in controlling hepatocyte behavior. Tgf-β is shown to suppress growth-inducing activity of various growth factors such as hepatocyte growth factor (HGF), epidermal growth factor (EGF) or insulin(Hayashi & Carr, 1985; Nakamura et al., 1985) . This growth-suppressive effect of Tgf-β depends on interaction between smad protein and Sp1 transcription factors leading to upregulation of cyclin-dependent kinase (CDK) inhibitor p21 (Moustakas & Kardassis, 1998). Besides being anti-proliferative, members of Tgf-β family of ligand were found to induce hepatocyte apoptosis (Oberhammer et al., 1991; Schwall et al., 1993; Yasuda et al., 1993).The pro-apoptotic property was shown to be mediated by enhanced expression of death-associated protein (DAP) kinase (Jang et al., 2002)and pre-apoptotic protein BIM (Ramesh et al., 2008). Tgf-β-mediated apoptosis is also involved death domain-associated protein (DAXX) that interacts with Fas receptor to induce the JNK activation (Perlman, Schiemann, Brooks, Lodish, & Weinberg, 2001).

In primary tumor, Tgf-β was found to have tumor-suppressive effects. This statement is supported by results from experiments using transgenic and knockout mouse models. One earlier report indicates that expression of dominant-negative mutant TβRII resulted in enhanced susceptibility to chemically induced hepatocarcinogenesis (Kanzler et al., 2001). Similarly, reduced expression of TβRII promoted the development of hepatocellular carcinoma induced by diethylnitroamine (Im et al., 2001). The increased incidences of HCC in the TβRII-deficient mice is associated with decreased Tgf-β-induced inhibition of hepatocyte growth and reduced expression of cell cylcle inhibitor, $p27^{kip1}$. In addition, it was shown that deletion of TβRII in hepatocytes leaded to increased proliferation up-regulation of Cdk2, Cyclin E and Cyclin A expression as well as down-regulation of Cdkn1a/p21 expression in HCC (Baek et al., 2010). These findings indicated tumor-suppressive effect of Tgf-β in the liver during hepatocarcinogenesis

5. Evidence for Tumor-Promoting Role of Tgf-β

While Tumor-suppressive effects of Tgf-β have been reported in the past decade, data from additional studies of mutant mice lacking TβRII in hepatocytes have challenged this idea. Baek et al., reported that abrogation of TβRII did not increase frequency or number of cancerous lesions found in mice overexpressing Tgf-α (Baek et al., 2010). Mu and Coworker also showed that the lack of TβRII did not promote diethylnitrosamine-induced hepatocarcinogenesis (Mu et al., 2016). These observations suggested that HCC cells might acquire resistance to its growth-inhibitory effect.

While the mechanism in which HCC uses to escape the tumor suppressive functions is still unknown, few speculations have offered to explain this effect so far. The study of HCC obtained from patient and rodent model of HCC showed that expression of TβRII was significantly lower in tumors when compared with the surrounding normal hepatocytes (Im et al., 2001; Mamiya et al., 2010).The reduced TβRII expression was associated with larger tumor size, poor differentiation, poor vein invasion, intrahepatic metastasis and shorter recurrence-free survival. These studies suggested that HCC might gain resistance to anti-proliferative effect of Tgf-β thru down-regulation of its receptor. It has also been shown that over-expression of oncogenic Ha-Ras suppressed

Tgf-β-induced inhibition of cell growth in hepatocytes (Houck, Michalopoulos, & Strom, 1989). Moreover, study showed activation EGFR signaling impaired cytostatic Tgf-β activity in preneoplastic setting (Dooley & ten Dijke, 2012; Murillo, del Castillo, Sanchez, Fernandez, & Fabregat, 2005). These data indicated that decreased Tgf-β-responsiveness in hepatocytes was at least in part mediated by EGF and Ha-Ras.

An increasing number of studies suggest that activation of Tgf-β promote HCC development. Transcriptome analysis of human HCC revealed that Tgf-β was associated with larger tumors and poor prognosis (Hoshida et al., 2009). In 25% of all early HCC, there is an association between Tgf-β signaling and expression of α-fetoprotein (AFP) and EpCAM (Yamashita et al., 2008). Tgf-β has been associated with increased T regulatory cells that provide immunosuppressive environment enabling tumor cells to escape immune response (Wolf, Sopper, Pircher, Gastl, & Wolf, 2015). Tgf-β was also shown to induce transformation of liver progenitor cells into tumor-initiating cells thru up-regulation of CD90 and CD133 expression (Majumdar et al., 2012; Wu et al., 2012). There is aaditional evidence obtained from genetic mouse models of HCC supporting the notion that Tgf-β is a tumor promotor. It was shown that disruption of TβRII reduces the formation of HCC in Tak1 knockout mice (Yang et al., 2013). Moreover, mutant mice lackin TβRII in hepatocytes have increased susceptibility to hepatocarcinogenesis induced by p53 loss (Morris et al., 2012).

It is becoming more evident that Tgf-β signaling is associated with highly invasive tumor condition. The contribution of Tgf-β in this stage of tumorigenesis was believed to be centered on the epithelial-to-mesenchymal transition (EMT) that is characterized by the loss of epithelial marker, such as, E-Cadherin and Cytokeratin, and by acquisition of fibroblast marker, such as, vimentin, collagen, fibronectin and α-smooth muscle actin (Guarino, Tosoni, & Nebuloni, 2009). It was shown that Tgf-β up-regulated of Snail transcription factor and induce EMT in HCC (Giannelli, Bergamini, Fransvea, Sgarra, & Antonaci, 2005). Inhibition of Tgf-β signaling pathway was shown to up-regulated E-Cadherin and block migration and invasion of HCC cells (Dituri et al., 2013; Fransvea, Angelotti, Antonaci, & Giannelli, 2008). In addition to acting as EMT-inducer, Tgf-β was known to promote HCC growth via angiogenesis regulation (Fransvea, Mazzocca, Antonaci, & Giannelli, 2009; Mazzocca, Fransvea, Lavezzari, Antonaci, & Giannelli, 2009). Moreover, Tgf-β was known to promote the cross-talk between tumor cells and stromal cells during tumor progression of HCC (Mazzocca et al., 2010).

6. Summary

Tgf-β has been shown to regulate several key steps in HCC tumorigenesis including proliferation, cell survival, migration, ECM production, angiogenesis and EMT. In pre-cancerous stage, Tgf-β seemed to make liver more susceptible to HCC development by enhancing HSC-mediated fibrosis and inflammation whereas it blocked malignant transformation of hepatocyte thru regulation of cell division and apoptosis. In certain neoplastic hepatocytes, an increasing rate of mutation in tumor cells leaded to activation of oncogenic gene that enabled tumor cells to escape the growth suppressive function of Tgf-β and acquire tumor promoting effect. Reversal of cytostatic effect of Tgf-β to tumor-promoting activity required up-regulation of secreting proteins and EMT-inducing factors that promote tumor environment. However, there are also evidence indicating that, in some situations, Tgf-β-mediated cytostatic activity was not disrupted and played an important role in suppressing HCC carcinogenesis. These finding suggest that one must be mindful in inhibiting Tgf-β signaling pathway in chronic liver damage. While targeting Tgf-β pathway may reduce fibrogenesis and inflammation, it may increase the risk of malignant transformation in hepatocytes with intact suppress function of Tgf-β. Future studies will aim to investigate the involvement of signaling molecules that modulate the cytostaic activity of Tgf-β during the initial transformation of HCC.

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