Modernization of Traditional Medicine

TGF-β signaling in hepatocellular carcinoma suppression and progression

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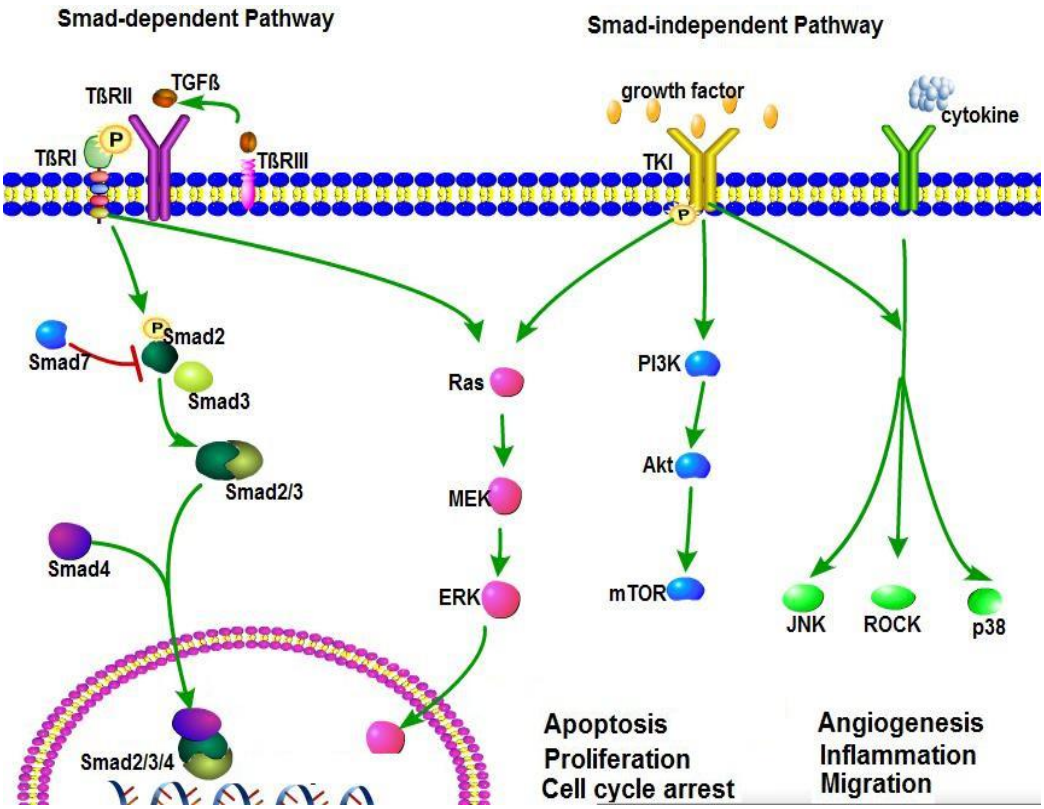
Highlights

This paper evaluates the suppressive and accelerant roles of TGF-β in hepatocellular carcinoma, discusses how a tumor-suppressor pathway can be so radically turned on its head and further provides some new molecular insights that may aid efforts towards targeted antitumor therapies.

Editor's Summary

This review pays particular attention to the dual role of TGF-β in hepatocellular carcinoma. It also discusses the potential anti-tumor herbs through TGF-β signaling pathways.



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Abstract

Derangements of several cell signaling pathways have been implicated in the initiation, progression, and development of hepatocellular carcinoma (HCC). One of such pathways is the activated TGF-β/Smad pathway. TGF-β inhibits proliferation and induces apoptosis in various cells types in the early tumor, and accumulation of loss-of-function mutations in the TGF-β receptor or Smad genes in tumor classify the pathway as a tumor suppressor. However, in chronic hepatitis, the cytostatic effect of TGF-β for hepatocytes attenuates as liver disease progresses from cirrhosis to HCC under persistent inflammatory microenvironments. In the later cancer period, TGF-β promotes tumor growth by modulating processes such as cell invasion, immune regulation, and microenvironment modification. Here we evaluate the suppressive and accelerant roles of TGF-β in HCC, discuss how a tumor-suppressor pathway can be so radically turned on its head and further provide some new molecular insights that may aid efforts towards targeted antitumor therapies. Moreover, we discussed the potential anti-tumor herbs through TGF-β signaling pathways.
Keywords: TGF-β, Hepatocellular Carcinoma, Suppression, Progression, Anti-tumor herbs

摘要

细胞信号通路紊乱在原发性肝细胞癌发生发展中发挥着不容忽视的作用。其中 TGF-β信号通路的活化在原发性肝癌中的作用引起广大学者的兴趣。在肿瘤早期，TGF-β抑制肿瘤细胞的增殖，诱导细胞凋亡，但随着肿瘤进展，肿瘤细胞内 TGF-β受体或其下游的 Smad 基因突变积累，其抑制作用减弱。在肿瘤晚期，TGF-β通过促进肿瘤细胞侵袭，参与免疫抑制，重建微环境基质，发挥着促进肿瘤进展的作用。因此，我们通过探讨 TGF-β在肝细胞癌中抑制作用和促进作用的机制，来进一步研究 TGF-β在肿瘤进展中，由抑制作用转化为促进作用的原因，为肿瘤进展和治疗提供新的思路。同时我们还论述了抗肿瘤草药在 TGF 信号通路中的作用。
关键词：TGF-β；肝癌；抑制；促进；抗肿瘤中药

Abbreviations: HCC, Hepatocellular carcinoma; EMT, Epithelial mesenchymal transition; DACH1, Dachshund homolog 1.
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Background

Hepatocellular carcinoma (HCC) is the most common liver malignancy and the third leading cause of cancer-related mortality worldwide. Current standard practices for treatment of HCC, surgical resection and chemotherapy are less than satisfactory because of metastasis and post-surgical recurrence [1]. Limited treatment options and delayed diagnosis caused by late occurring symptoms highlight the urgent need to characterize the heterogeneity of oncogenic mechanisms in HCC and to identify early disease biomarkers and new targets.

In the last decades, researches have provided significant insights into TGF-β signaling pathway as its misregulation can result in tumor development. The regulatory cytokine TGF-β exerts tumor-suppressive effects that cancer cells must elude for malignant evolution. Yet, paradoxically, TGF-β also promotes advanced tumor growth by modulating processes such as cell invasion, immune regulation, and microenvironment modification. TGF-β exerts pleiotropic effects in human liver cells. It is prominent in damaged liver and represents a key regulator of hepatic stellate cell activation and liver fibrogenesis upon most types of liver damage [2]. It displays cytostatic effects inducing apoptosis in distinct hepatocytes and interfering with hepatocyte proliferation during liver regeneration [3]. During the process of chronic liver damage progression towards cirrhosis and HCC, TGF-β is assumed to switch from cytostatic to oncogenic action on hepatocytes and becomes a plasticity factor that induces epithelial mesenchymal transition (EMT), cytokine and receptor production, migration and invasion. This review pays particular attention to the dual role of TGF-β in hepatocellular carcinoma.

Mechanisms of TGF-β signaling

TGF-β family and TGF-β receptors

The human TGF-β family comprises more than 30 factors. There are three different TGF-βs, TGF-β1, TGF-β2 and TGF-β3 [4]. Of these, TGF-β1 is the most frequently upregulated in tumor cells [5]. The human TGF-β family regulates the establishment of the body plan and tissue differentiation through their effects on cell proliferation, differentiation and migration [6]. In this review, we primarily discuss TGF-β, and wherever possible. We refer to other members of the super family.

There are seven type I receptors and five type II receptors paired in different combinations to provide the receptor system for the entire TGF-β family. Most members of the TGF-β family share several type I and type II receptors, but TGF-β is an exception. Only TβRII can bind to TGF-β and only TβRI can be incorporated into this TβRII – TGF-β complex [7].

The cytoplasmic region of these receptors contains a serine/threonine kinase domain. In the N-terminal to the kinase domain of type I receptors, a short segment (the GS domain) provides a switch for kinase activation. Ligand dependent phosphorylation by a type II receptor switches the GS domain from a repressor element into a docking site for substrate Smad proteins [8].

Various membrane proteins enhance binding of ligands to the receptors [8]. The so called TGF-β type III receptor is the membrane-anchored proteoglycan betaglycan. It binds and presents TGF-β to the TGF-β type II receptor. Some proteins (ligand traps) trap TGF-β to limit their access to membrane receptors and the overexpression of protein follistatin is implicated in hepatocarcinogenesis [9, 10].

Smad-dependent and Smad-independent pathways in TGF-β family signaling

The bioactive TGF-βs molecule is a dimmer composed of a polypeptide chain that is produced upon proteolytic cleavage of the latent disulfide-linked homodimeric polypeptide. On binding to dimeric TGF-β, the type II receptors and the type I receptors form hetero-tetramic complexes. In the receptor complex, type II receptors trans-phosphorylate and activate type I receptors that then propagate the signal by phosphorylating Smad transcription factors. Receptors of the TGF-β branch of the cytokine family phosphorylate Smads 2 and 3. Once activated the receptor substrate Smads (RSmads) shuttle to the nucleus and form a complex with Smad 4, a binding partner common to all R-Smads [7]. Then each Smad4-RSmad-cofactor combination additionally recruits transcriptional coactivators, corepressors, and chromatin remodeling factors to activate or repress hundreds of target genes at once. Except the mentioned canonical TGF-β/Smad signal pathways, the Smad-independent pathways are supported by the identification of the Erk, JNK and p38 MAPK kinase pathways [11-17]. The TGF-β signaling pathway has been shown in Figure 1.

TGF-β tumor suppression mechanisms in HCC

Cytostastics mechanism

TGF-β inhibits progression of cell cycle phase G1 through two sets of events: mobilization of cyclin-dependent kinase inhibitors and suppression of *c-MYC*[18]. Heterozygous mice with reduced TGF-β expression results in enhanced susceptibility to HCC, confirming the tumour-suppressor function of the TGF-β signaling pathway [19]. In hepatocytes, TGF-β acts as a principle growth inhibitor, mediated by inducing expression of cyclin-dependent kinase inhibitors p21 and p15, and down-regulating c-MYC, Cyclin D, and Cyclin E [20, 21].

Proapoptotic mechanisms

Inherent to the tumor suppressor action of TGF-β is its ability to induce apoptosis. TGF-β induces apoptosis in several established human liver cell lines, including HepG2 hepatoma and HepG3 HCC cells [22]. Smad signaling regulates expression of several apoptotic genes. These genes include *DAPK* (death-associated protein kinase), *GADD45β*, *BIM* and *SHIP*. GADD45β interacts with and activates the mitogen-activated protein kinase

kinase 4 (MKK4), which then activates the MAPK p38, leading to caspase-8 and Bad activation. In hepatoma cells, the signaling factor GADD45β triggers apoptosis [23-25]. In rat hepatoma cells, SMAD3 is proved to mediate the caspase-dependent cleavage of BAD [26].

Other pathways also lead to apoptosis in response to TGF-β, including MAPKs, such as p38 and c-Jun N-terminal kinase (JNK). In the apoptosis of hepatocytes, TβRII directly associates with the Fas receptor adaptor protein DAXX, which then activates JNK activation [27]. Alternatively, the mixed lineage kinase MLK3 can also be the upstream activator of p38 in response to TGF-β in apoptosing hepatoma cells [28].

In hepatoma cells, TGF-β1-induced apoptosis is also mediated by decreased phosphorylation of CDC2 at Tyr15 accompanied by down-regulation of Wee1 kinase expression. Wee1 kinase was expressed in moderately to poorly differentiated HCC, whereas no Wee1 kinase expression was observed in non-cancerous tissue, including cirrhotic tissue [29]. Downstream gene of the Notch and TGF-β pathways, hairy/enhancer-of-split related with YRPW motif-like (HEYL) protein also caused apoptosis in HCC. However, HEYL expression was inactivated in more than 75% of HCC by DNA hypermethylation which directly correlated with the progression of HCC [30]. NF-kappaB and antioxidants can counteract TGF-beta1-induced apoptosis in malignant hepatocytes through JNK1 pathway up-regulation of its downstream genes, such as X-linked inhibitor of apoptosis protein (XIAP) [31].

Cell senescence and autophagy

TGF-β and other members of its family have a major influence on cell senescence. TGF-β induces p53-independent and p16 (Ink4a)-independent, but Nox4-dependent, p21 (Cip1)-dependent, p15 (Ink4b)-dependent, and ROS-dependent senescence arrest in well-differentiated HCC cells. Moreover, TGF-β-induced senescence in vivo is associated with a strong antitumor response against HCC [32]. TGF-β induces autophagy in hepatocellular carcinoma cells and mammary carcinoma cells. The autophagy pathway might contribute to the growth inhibitory effect of TGF-β, in conjunction with other anti-proliferative pathways downstream of TGF-β signaling. Autophagy activation by TGF-β is mediated through the SMAD and JNK pathways.

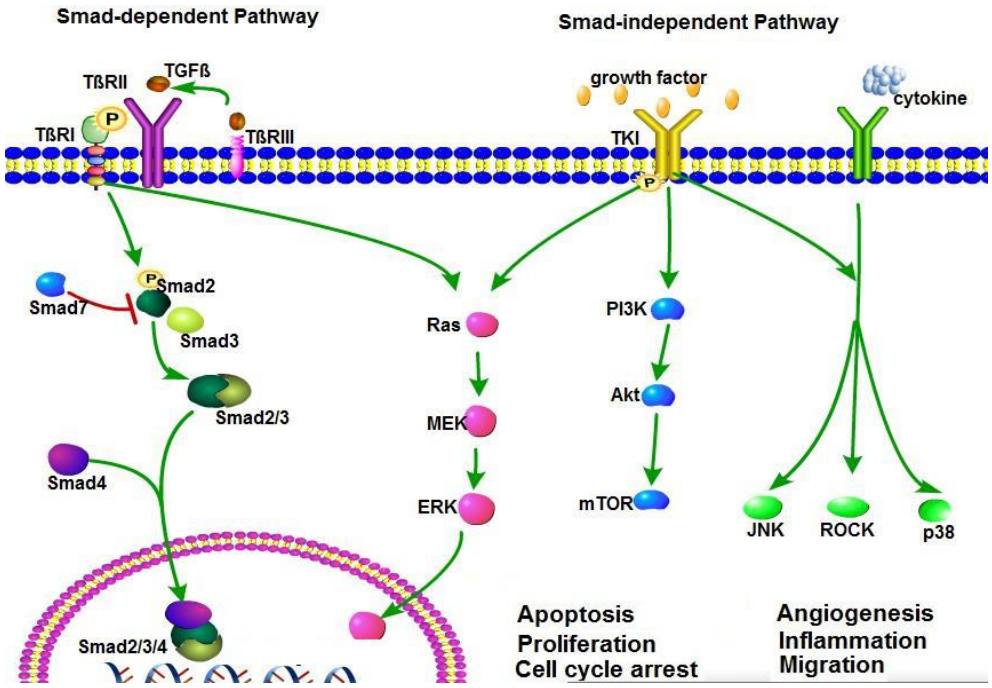

Figure 1 Smad-dependent and Smad-independent TGF-β pathways

In the Smad-dependent pathway, the three TGF-β ligand isoforms, TGF-β1, TGF-β2, and TGF-β3, are activated and assisted by the membranous TGF-β type III receptor to bind to TGF-β type II receptor (TGF-βRII) with high affinity. TGF-βRII binding allows dimerization with TGF-β type I receptor (TGF-βRI) homodimers, activation of the TGF-βRI kinase domain and signal transduction via phosphorylation of the C-terminus of receptor-regulated SMADs (R-SMAD), SMAD2 and SMAD3. The TGF-βR dimer then forms a heterotrimeric complex with SMAD4 which translocates and accumulates in the nucleus. TGF-β dependent signaling can activate or repress hundreds of target genes through the interaction of SMADs with various transcription factors (TF). SMAD activities are regulated via expression of inhibitory SMAD6 and SMAD7. In the Smad-independent pathway, TGF-β signaling activates other pathways such as PI3K/AKT, MAPK pathways (ERK, JNK, and p38 MAPK) as well as NF-kB, Rho/Rac1, CDC42, FAK, Src, Abl. The TGF-β signaling pathway has pleiotropic functions regulating cell growth, differentiation, apoptosis, cell motility, extracellular matrix production, angiogenesis and cellular immune response.

SiRNA-mediated knockdown of autophagy genes suppresses the growth inhibitory function of TGF-β and that autophagy activation potentiates TGF-β-mediated induction of proapoptotic genes, *BIM* and *BMF*, in hepatoma cells [33].

Inhibit tumor angiogenesis

Consistent with a tumor suppressor function, TGF-β inhibits angiogenesis and results in reduced tumor growth. In hepatoma cells, mutant TGF-β that represents only the extracellular, soluble domain of this receptor, which binds TGF-β with high affinity, results in inhibition of autocrine TGF-β activity and subsequent secretion of VEGF. Thus, blocking autocrine TGF-β induces expression of VEGF, which results in significant angiogenic effects within the tumors that these hepatoma cells develop upon implantation into mice [34].

TGF-β cancer progression in HCC

Currently the pro-tumorigenic role of TGF-β can be explained by a complex set of cellular mechanisms. On the one hand, malignant cells failure to response to TGF-β-suppressive mechanisms through inactivation of core components of the pathway, such as TGF-β receptors, Smad4, or by losses of the tumor-suppressive arm of the TGF-β. On the other hand, TGF-β modulates progresses such as microenvironment modification, cell invasion, immune regulation, and regulation of tumor angiogenesis that cancer cells may exploit to their advantage. The following will pay particular attention to insights that are relevant to HCC. The related mechanisms have been summarized in Figure 2.

Malignant cells can circumvent the suppressive effects of TGF-β either through inactivation of core components of the pathway, or by downstream alterations. In agreement with this notion, elevated levels of TGF-β1 mRNA have been reported in HCC, and TGF-β1 is also increased in the plasma of patients with HCC [35, 36]. Furthermore, the loss of responsiveness of HCC cells to TGF-β1-mediated growth inhibition has been implicated in hepatocarcinogenesis [37].

Mutations in the TGF-β superfamily genes have been found in various cancers. The TGF-β receptor II (TβRII) gene is frequently mutated in colon cancers, gastric cancers, and in gliomas with microsatellite instability [38-41]. In HCCs, however, mutations in TGF-β superfamily genes are rare [42, 43]. Some reports show in HCC TβRII expression downregulation in 50-60% compared with adjacent liver tissue [44]. Reduced TβRII expression correlated with portal vain invasion in HCC cases, and poorly differentiated HCC cells [45]. The reduced availability of TGF-β type II receptor (TβRII) by the ectopic expression of soluble TβRII in hepatocytes also has been found to abrogate the cytostatic effect of TGF-β on hepatocytes [46].

Recent studies have emphasized the possibility of the Smads family's involvement in the pathogenesis of HCC diseases. The reversible shifting of SMAD3-mediated signaling between tumor suppression and oncogenesis has been discovered [47]. Activation of SMAD3 with TGF-β binding and Ras-related kinase, including JNK and CDK, differentially phosphorylate SMAD3 to form pSMAD3C and the pSMAD3L. These two domain-specific phosphorylation forms have different actions. The pSMAD3C transmits a tumor-suppressive TGF-β signal, while pSMAD3L promotes its oncogenic activities.

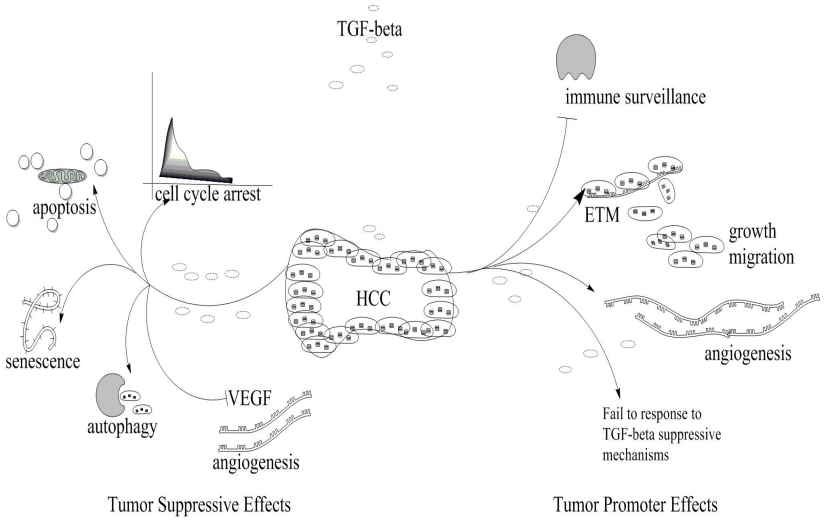

Figure 2 TGF-β signaling in HCC progression and suppression

Tumor suppressive effects. Cell cycle arrest by increasing tumour suppressing genes and decreasing oncogenes; Induces autophagy, differentiation, senescence and apoptosis; Suppresses angiogenesis through inhibiting VEGF; Inhibits inflammatory cytokine production from lymphocytes and macrophages.

Tumor promoter effects. Growth and migration promotion of HCC cells via inducing the production of growth factors epithelial-mesenchymal transition induction, evasion of immune surveillance, Augments microenvironment-modifying proteases and cytokines; invasion and angiogenesis by inducing expression of genes such as *MMP2*, *MMP9* and *CTGF*.

In HCC, virus components, including the HBXprotein; inflammatory cytokines such as IL-1β and TNF-α; growth factors acting through a tyrosine kinasetype receptor, including hepatocyte growth factor and platelet-derived growth factor (PDGF); and ras mutations additively upregulate phosphorylation of SMAD3L by activated JNK MUC1 overexpression also enhanced the levels of p-SMAD3L (Ser-213) and its target gene *MMP-9* in HCC cells [48-50].

The most frequently mutated *SMAD* gene in human cancer is *SMAD4*, which is originally discovered as a novel tumor suppressor gene of chromosome 18q21.1 and is corrected with abolishment of TGF-β suppressions. Nuclear SMAD4 4 levels are significantly increased in patient HCC tumors as compared with adjacent tissues. Knockdown of SMAD4 significantly reduced the efficiency of colony formation and migratory capacity of HCC cells in vitro and was incompatible with HCC tumor initiation and growth in mice [51]. SMAD4 intragenic mutations also have been mapped in HCC, and Smad4 mutations are selected at late stages of carcinogenesis and their frequency increases as tumors progress towards metastasis [52].

Loss of SMAD2 function appears sufficient in inhibiting the physiological TGF-β signaling pathway. In liver cancers, a number of inactivating missense or nonsense point mutations have been mapped primarily in the MH2 domain and less frequently in the MH1 domain of SMAD2 [52, 53]. These point mutants result in protein instability, thus creating rapidly degrading SMAD2 variants [54].

The expression of SMAD5, one of the other branches of TGF-β family BMP signaling pathway R-SMADs, is found elevated in HCC. SMAD5 has also been proposed to mediate signals by TGF-β [55].

The inhibitory SMAD6 and SMAD7 attenuate normal TGF-β and BMP signaling, and accordingly the prediction has been that these proteins might be overexpressed in human tumors that acquire resistance to TGF-β-mediated growth inhibitory responses. However, in HCC, failure to identify I-Smad mutations or aberrations in expression has been reported [56].

Moreover, the mutations of RAS and c-MYC pathways are discovered blocking TGF-β-suppression. Ectopic expression of mutated Ha-Ras has been found to abrogate the cytostatic effect of TGF-β on hepatocytes [57]. Accordingly, in liver cancers, hyperactive β-catenin/LEF/TCF signaling leads to sustained expression of c-MYC; this secondarily makes cells unable to elicit growth arrest in response to TGF-β [58]. ELF(embryonic liver fodrin), a spectrin family scaffolding protein, interacts with phosphorylated SMAD3 and with SMAD4 and promotes their nuclear translocation, while loss of ELF in mouse knockouts results in defective TGF-β/Smad signal transduction [59]. ELF function within the TGF-β pathway seems to explain why expression of this protein is lost in human liver cancers [60, 61].

Inactivation of TP53 and inhibition of TGF-β signaling are among the most common molecular events in human liver cancers [62].

Human dachshund homolog 1 (DACH1) is a major component of the Retinal Determination Gene Network (RDGN) and functions as a tumor suppressor. DACH1 suppressed cellular growth by reactivating TGF-β signaling. Down-regulation of DACH1 is a novel mechanism for gaining resistance to the antiproliferative signaling of TGF-β1 in HCC cells. Ectopic expression of DACH1 enhanced chemosensitivity to 5-fluorouracil (5-FU) by inducing p21 expression in HCC cells. Promoter region hypermethylation was correlated with loss or reduction of DACH1 expression in HCC cell lines. Promoter region methylation was found in 42% of primary HCC. Reduced expression of DACH1 was associated with poor differentiation of HCC nodules and higher serum aspartate aminotransferase/alanine aminotransferase ratio [63].

MiR-183 transfectants are resistant to apoptosis induced by TGF-β. MiR-183 targets the gene of PDCD4, which is a proapoptotic molecule involved in TGF-β1-induced apoptosis in human HCC cells. MicroRNA-183 was significantly up-regulated (twofold to 367-fold) in compared with the matching nontumoral liver tissues [64].

Direct tumorigenic effects of TGF-β in HCC

Pro-tumorigenic role of TGF-β is associated with TGF-β-induced production of autocrine mitogens. Moreover, TGF-β also modulates processes such as epithelial mesenchymal transition (EMT), cell invasiveness, the mobilization and function of myofibroblasts, neoangiogenesis and microvessel formation, and immune regulation, which are summarized as following.

TGF-β induces production of several mitogenic factors such as hepatocyte growth factor/scatter factor or platelet derived growth factor by which TGF-β can promote tumor cell proliferation [65].

EMT and cell motility during carcinoma progression emphasizes the role of the tumor microenvironment. EMT is a pathological feature in neoplasia and fibrosis [66]. TGF-β is a potent inducer of EMT, and in human carcinomas, cells with features characteristic of EMT have been observed in the invasion front, a location that is rich in stromal TGF-β and other cytokines that may cooperate in EMT induction [67, 68]. Indeed, upon activation of the TGF-β pathway, carcinoma cells that exhibit more over overt and irreversible EMT lead to more aggressive and metastatic tumors [69]. EMT in response to TGF-β1 is characterized by down-regulation of epithelial markers such as E-cadherin, specific keratins, and ZO-1, and upregulation of mesenchymal markers such as Fibronectin, FSP1, α-Smooth muscle actin and Vimentin [70, 71]. The competence of epithelial precursor cells to undergo EMT becomes manifest cues in response to TGF-β [72]. Both SMAD and non-SMAD signaling have been activated in TGF-β-induced EMT in tumors. TGF-β1, TGF-β2, TGF-β3 can induce EMT in vitro and in vivo by activating SMAD2 and SMAD3, while the BMPs inhibits TGF-β from eliciting EMT

[73-76]. In hepatocarcinomas, it has been shown that connective tissue growth factor, a protein regulated by TGF-β, acts as a fundamental regulator of fibrogenesis leading to the accumulation of extracellular matrix proteins in the liver and kidney [77, 78]. It also has been established that oncogenes such as Ras or Raf induce EMT and tumor cell invasiveness in a TGF-β-dependent manner [79-81]. Reichl P has shown the up-regulation and activation of the receptor tyrosine kinase AXL in EMT-transformed hepatoma cells. Knockdown of AXL expression resulted in abrogation of invasive and transendothelial migratory abilities of mesenchymal HCC cells in vitro and AXL overexpression-induced metastatic colonization of epithelial hepatoma cells in vivo [82]. Myocyte enhancer factors 2 family proteins were found to be overexpressed in HCC cells under the treatment of TGF-β1 in a PI3K/Akt-dependent way. Silencing the expression of MEF2s was able to prevent the effect of TGF-β1 on HCC EMT and invasion [83]. The tetraspan (also called tetraspanin) TM4SF5 (transmembrane 4 L6 family member 5) is highly expressed in hepatocellular carcinoma and induces EMT, TGFβ1 and growth factor-mediated signaling activities mediate TM4SF5 expression leading to acquisition of mesenchymal cell features, suggesting that TM4SF5 induction may be involved in the development of liver pathologies [84]. In vitro studies have proposed a tumor suppressor role for sulfatase 1 in hepatocellular carcinoma. However, high expression in human HCC has been associated with poor prognosis. Overexpression of sulfatase 1 promotes TGF-β-induced gene expression and EMT and enhances cell migration/invasiveness [85]. CD44s also plays a critical role in the TGF-β-mediated mesenchymal phenotype and therefore represents a potential therapeutic target for HCC [86].

An immediate corollary to EMT is the process of cancer cell invasiveness, in which cell motility and extracellular milieu degradation are required. TGF-β secreted from carcinoma cells best correlates with the invasive and metastatic properties of the tumor. The action of TGF-β is more important locally as a modulator of tumor microenvironment. Pharmacologic treatment of such carcinomas with a specific inhibitor of TβRI/ALK5 kinase blocked in vitro motility and invasiveness [87, 88]. The cellular mechanisms that explain the motile and invasive phenotype in response to TGF-β are diverse and include both SMAD-dependent gene regulation and activation of alternative signaling effectors in the carcinoma cell. In HCC cells, the expression of α3β1-integrin which induced by TGF-β is involved in motility and invasiveness which depend critically on the level of the integrin receptor in these cells [89].

The mobilization of myofibroblasts is another significant component of the proinvasive action of TGF-β, which stimulates the generation of myofibroblasts from mesenchymal precursors [90]. Myofibroblasts are the called “cancer-associated fibroblasts” and produce matrix metalloproteases, cytokines and chemokines to promote cancer cell proliferation, tumor invasion and neoangiogenesis [91]. TGF-β drives the progress of invasion by acting on the myofibroblasts which is correlated with TGF-β-stimulated JNK pathway and the expression of N-cadherin [92]. TGF-β1 continuously acts on myofibroblasts, and induces expression of HGF/SF, which promotes carcinoma proliferation and invasion [93].

Another functional consequence of tumor growth factor activity in the tumor microenvironment is the induction of neoangiogenesis and microvessel formation. HCC overproduce TGF-β in vivo and the higher the level of TGF-β the higher degree of neovascularization observed in these tumors and the higher their chance for metastasis, and the high level of TGF-β correlates with loss of TβRII expression, the degree of malignancy, tumor invasiveness and metastatic potential [94-97]. The positive role of TGF-β on angiogesis is supported by the nude mice model which has faster tumor growth and massive angiogenesis inside the tumor mass with transfected TGF-β. In vitro studies established that TGF-β plays as a promoter of angiogesis via induction of VEGF [98].

A very important function of TGF-β in the tumor stroma is the modulation of immune cells. TGF-β acts as an inhibitor of B or T lymphocyte proliferation and differentiation, resulting in the most potent natural immunosuppressor in the human body. TGF-β deactivates scavenging macrophases and thus protects the developing tumor from proper immune surveillance [99]. Hepatitis B virus-related chronic liver disease is the most important risk factor for development of HCC. Importantly 50-60% of HCC in Asia is associated with chronic HBV infection [100-102]. During chronic hepatitis B infection, the increased regulatory cells (Tregs) exert their suppressive effects either via cell to cell contact by membrane-bound molecules or through contact-independent manner mainly by release of IL-10 and TGF-β cytokines [103]. TGF-β and IL-10 are responsible for the suppression of anti-tumor immune responses and therefore lead to successful tumor escape [104].

The potential anti-tumor herbs through TGF-β signaling pathways

Actions of many herbal medicine products for cancer treatment are linked to TGF-β in the target cells. Since TGF-β is growth inhibitory and can induce apoptosis in normal non-cancer target cells, such property will be a suitable anti-cancer supplements for cancer prevention. Examples for these products include seaweed and resveratrol [105, 106]. Other products, such as Long Dan Tan, also have the ability to induce TGF-β production in target cells [107, 108]. It should be pointed out that, since these agents can induce TGF-β production in target cells, they are suitable for cancer prevention but should be careful in administrating these agents for the purpose of treatment of established cancers. It is important to note that many natural products that can induce TGF-β production in the target cells may be beneficial in preventing cancer development but may be harmful for cancer patients, especially when they harbor advanced

stage cancer.

In cancer target cells, inhibiting TGF-β signaling could help to inhibit tumor growth and metastasis. The best examples of this class of herbal products are flavenoids, such as genistein, with their ability to inhibit tumor progression and metastasis [109]. Zhuzicao (*Phyllanthus niruri L*). is a well-known hepatoprotective and antiviral medicinal herb. Corilagin extracted from Zhuzicao (*Phyllanthus niruri L*). acts as an effective therapeutic agent against the growth of cancer cells via targeted action against the TGF-β/Akt/Erk/SMAD signaling pathways [110]. Other natural products which could inhibit the TGF signaling pathway were green tea and black tea extracts, angelica sinensis, machilus thunbergii, Chunggan extract, esculentoside A, rhubarb extract, compound astragalus and salvia miltiorrhiza extract, momordica charantia leaf extract, and polypodium leucotomos [111-123].

Conclusion

In this review, we outlined the major functions of TGF-β in HCC. The current evidence suggests that it is possible to change between tumor suppressors to pro-tumorigenic effects of TGF-β. Therapies which target TGF-β should base on tumor stages. Therefore, effective molecular markers for suppressive or progressive functions should be further searched to provide evidence for TGF-β-targeted therapy.

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