76266_Auto_Edited-check.docx

WORD COUNT	4438	TIME SUBMITTED	05-AUG-2022 01:25PM
		PAPER ID	89227575

Name of Journal: *World Journal of Gastroenterology*
Manuscript NO: 76266
Manuscript Type: REVIEW

Deciphering the role of transforming growth factor-beta 1 as a diagnostic-prognostic-therapeutic candidate against hepatocellular carcinoma

Devan AR *et al*. Deciphering role of TGF-β1 against HCC

Abstract

Transforming growth factor-beta (TGF-β) is a multifunctional cytokine that performs a dual role as a tumor suppressor and tumor promoter during cancer progression. Among different ligands of the TGF-β family, TGF-β1 modulates most of its biological outcomes. Despite the abundant expression of TGF-β1 in the liver, steatosis to hepatocellular carcinoma (HCC) progression triggers elevated TGF-β1 levels, contributing to poor prognosis and survival. Additionally, elevated TGF-β1 levels in the tumor microenvironment create an immunosuppressive stage *via* various mechanisms. TGF-β1 has a prime role as a diagnostic and prognostic biomarker in HCC. Moreover, TGF-β1 is widely studied as a therapeutic target either as monotherapy or combined with immune checkpoint inhibitors. This review provides clinical relevance and up-to-date information regarding the potential of TGF-β1 in diagnosis, prognosis, and therapy against HCC.

Key Words: Transforming growth factor-beta 1; Inflammation; Immunosuppression; Fibrogenesis; Hepatocellular carcinoma; Biomarker; Immunotherapy

Devan AR, Pavithran K, Nair BL, Murali M, Nath LR. Deciphering the role of transforming growth factor-beta 1 as a diagnostic-prognostic-therapeutic candidate against hepatocellular carcinoma. *World J Gastroenterol* 2022; In press

Core Tip: Transforming growth factor-beta 1 (TGF-β1) exhibit a progressive elevation throughout hepatic dysfunction starting from hepatitis to hepatocellular carcinoma (HCC) as an inflammatory cytokine, pro-fibrogenic marker, immunosuppressive agent and pro-carcinogenic growth factor. Aberrant TGF-β1 activation in HCC is associated with poor prognosis and survival. TGF-β1 mediated immunosuppression disturbs the anticancer surveillance and the efficacy of the immunotherapeutic agent. This pleiotropic effect of TGF-β1 in the context of HCC makes it ideal as a diagnostic, prognostic, and therapeutic candidate in HCC.

INTRODUCTION

Hepatocellular carcinoma, an aggressive and refractory cold tumor

Liver cancer, specifically hepatocellular carcinoma (HCC) is often recognized as an aggressive malignancy, ranking 6th in incidence and 3rd in terms of mortality in 2020, where mortality rates are roughly equivalent to incidence rate[1]. As it develops in the background of chronic inflammation starting from the fatty liver, HCC remains undiagnosed for years until it worsens. The progressive transformation from cirrhosis to HCC also creates longer delays in diagnosis[2]. HCC is commonly diagnosed by liver imaging techniques such as ultrasound, computed tomography and magnetic resonance imaging. Blood biomarkers such as alpha feto protein (AFP), protein induced by vitamin K absence or antagonist II levels, and liver biopsy are also used to diagnose HCC[3,4]. Even though the recent guidelines recommend HCC surveillance biannually and antiviral vaccination, the lack of effective surveillance programs significantly contributes to HCC progression to the advanced stage, particularly in high-risk individuals. Once established, HCC cells rapidly proliferate and spread to the extrahepatic site, such as the lungs, portal vein, and lymph nodes. In such cases, in an advanced stage, curative interventions such as liver transplantation, resection, percutaneous ablation, and chemoembolization were not responsive. Systemic drug therapy remains the primary treatment modality[5,6], where immunotherapy and tyrosine kinase inhibitors are the approved treatment options[7]. However, limited response to therapy, the emergence of multidrug resistance, immunosuppressive tumor microenvironment, and lack of validated diagnostic and prognostic biomarkers pose significant obstacles in establishing effective treatment against HCC[8,9].

Transforming growth factor-beta (TGF-β) is a critical homeostasis regulator, which is aberrantly activated during inflammation, fibrosis and carcinogenesis[10]. Among the three isoforms, TGF-β1 is superior in TGF-β signal transduction, especially those related to chronic liver diseases. Since HCC is an inflammation-induced-immunosuppressed malignancy, the role of TGF-β1 signaling in HCC has been extensively evaluated

recently[11]. This review presents the potential of the TGF-β superfamily of ligands, specifically TGF-β1, to develop as a therapeutic and prognostic-diagnostic marker candidate against HCC.

TGF-β1 signaling in HCC

TGF-β superfamily of ligands is a dimeric peptide growth factor with more than 30 members in humans, mainly TGF-βs, activins, inhibins, and bone morphogenetic proteins. TGF-βs are categorized into three different isoforms TGF-β1, TGF-β2, and TGF-β3. Of the three isoforms, TGF-β1 is the most evaluated and abundant, found in epithelial, endothelial, hematopoietic, and connective tissue. TGF-β 2 is expressed in epithelial and neuronal cells, while TGF-β3 is found in mesenchymal cells[12]. TGF-βs are implicated in diverse physiological processes, including cell homeostasis and embryonic development[13]. It is a pleiotropic factor that regulates inflammation, fibrogenesis, cell differentiation, proliferation, epithelial-mesenchymal transition (EMT), extracellular matrix (ECM) formation, tumor-suppressive and pro-tumor effect in a cell-context dependent manner[14].

The three isoforms of TGF-β have structural similarity and functional redundancy[15]. However, TGF-β1 is often considered a potent and superior isoform with significant physiological and pathological importance[16]. Importantly, TGF-β1 exerts a cell-context dependent effect in the liver[17,18]. Normal to activated TGF-β1 signature confers a protective effect by inhibiting hepatocyte proliferation and hepatic stellate cells (HSCs) activation, apoptosis induction, preventing fibrosis and improving liver function. While, aberrantly activated TGF-β1 signature manifests as HSC activation and worsening fibrosis to HCC, where tumor cells lose their sensitivity toward the inhibitory effect of TGF-β1[19-21]. Exposure of hepatocytes to various causative factors such as viruses, alcohol, toxicants and other metabolic disorders leads to the release of TGF-β1. Also, other pro-inflammatory cytokines such as tumor necrosis factor-α and growth factors from non-parenchymal liver cells switch on inflammation, production of ECM, and accumulation of fibrous material that eventually progress to cirrhosis[22,23]. A

simultaneous increase in integrins, a vital cell adhesion molecule, is observed as the fibrogenesis continues. These integrins interact with TGF-β1 and other ECM proteins, altering signal transduction pathways[24]. Along with the accumulation of genetic mutations, HSC induces TGF-β and β-catenin-dependent EMT, leading to tumor growth in the liver. TGF-β1 continues to increase, promoting neo-angiogenesis by interacting with other pathways and mediating stromal-tumor cell interaction, conferring aggressive phenotype and metastasis[25] (Figure 1). Wang *et al*[26] demonstrated that both TGF-β1 and TGFβR1 have a crucial role in regulating proliferation, invasion, metastasis and immune response in HCC cells.

TGF-β1 exerts biological and pathological effects *via* Smad and non-Smad pathways. TGF-βs are synthesized in the inactivated form and exist as latent TGF-β complex (LTC) by binding with latency-associated protein. Later, LTC is converted to large latent complex (LLC) by interacting with latent TGF-β binding protein in ECM. Integrin signaling plays a significant role in the activation and subsequent release of TGF-β1 from LLC. It is also mediated by other factors such as pH, protease enzyme *etc.*[27]. In the canonical Smad pathway, activated TGF-β first binds with the extracellular domain of TGF-β receptor type II, which triggers the cross phosphorylation of the kinase domain of TGF-β receptor type I. TGF-β R1 activation leads to the phosphorylation of Smad proteins, Smad 2 and Smad 3. Later Smad-2 and 3 complex bind with the co-Smad, Smad-4 to form a ternary complex. This ternary complex is then translocated into the nucleus, binds to Smad binding elements in DNA, and activates the transcription of TGF-β-dependent genes[28]. Binding of inhibitory Smad, Smad-7 will shut down the activated pathway[29]. In addition to the Canonical Smad pathway, TGF-β can exert biological functions by activating other diverse signalings such as P38, JNK, PI3K/AKT, RAS-ERK, and RHO-ROCK, which constitute the non-Smad pathway of TGF-β signal transduction[30] (Figure 2).

Smad pathway of TGF-β signal transduction also enhances the transcription of FoxP3, predominantly present in T regulatory (Treg) cells[31,32]. A high amount of tumor-infiltrating Treg cells and FoxP3 positive Treg cells in blood is reported in HCC

patients, leading to the deterioration of effector T cells such as CD4+ and CD+ cytotoxic T cells lymphocytes, which are pillars of anticancer immunity[33,34]. Together, TGF-β inhibits natural killer (NK) cells, blocks interferon (IFN)-γ secretion, and prevents effector immune cells recruitment to tumor tissue[35,36]. Additionally, TGF-β inhibits IFN-γ secretion by interacting with the activating transcription factor 1[37]. Likewise, TGF-β-RUNX3 transcription factor interaction and co-expression of programmed death ligand 1 (PD-L1) and interleukin-10 promote the transformation of naïve B cells to immunoglobulin A producing B cells, which are crucial in HCC development from non-alcoholic fatty liver[38]. Elevated TGF-β can directly enhance the transcription of PD-1 in HCC. The interaction of PD-1 with PD-L1 causes significant immunosuppression by T cell exhaustion, which manifests as inhibition of T cell activation, proliferation, and cytotoxic action[39,40]. In a recent study, Bao *et al*[41] reported that TGF-β1 trigger the expression of immune checkpoints such as PD-1 and CTLA4 on HCC cells and attenuates T-cell-mediated anti-tumor immune surveillance. Therefore, up-regulated TGF-β mainly isoforms 1 directly affect immune checkpoint inhibition, and it works as an indicator of T cell exhaustion. This evidence suggests the potential of TGF-β1 targeted immunotherapies against HCC. The pivotal role of the TGF-β1 signature in hepatic dysfunction and HCC extends its potential as a biomarker molecule for diagnosis and prognostic prediction and a therapeutic target.

Clinical utility of TGF-β1 as diagnostic marker of HCC

As an inflammatory-fibrogenic cytokine molecule, the involvement of TGF-β1 in all stages of liver injury, starting from fatty liver, steatosis, fibrosis to cirrhosis, and HCC, is evident. Intergromic analysis of TGF-β gene alterations among the 33 cancer types in the TGCA dataset revealed 39% alterations. Gastrointestinal cancers and HCC exhibited prominent mutation compared with other cancer types[42]. Later, in an HCC-specific transcriptomic analysis, 40% of HCC samples were found with mutations in genes of the TGF-β pathway[43]. Higher TGF-β1 levels in HCC correlate with the high rate of extrahepatic metastasis (EHM), poor prognosis, and low survival rate[44]. After

acute/chronic liver injury, liver sinusoidal endothelial cells and HSCs secrete TGF-β1 and up-regulate TGF-β receptors[45,46]. Elevated TGF-β1 level was found in viral and alcohol-induced fibrosis[47]. Thus, TGF-β levels can be used to track the response to therapy so that the decrease in TGF-β 1 level followed by IFN treatment in hepatitis B virus (HBV) patients is associated with improved treatment outcomes. Apart from HSC-triggered TGF-β secretion, hepatitis C virus (HCV) infection can also induce TGF-β1 production in hepatocytes[48]. Likewise, proteomic and phospho-proteomic characterization of 110 tumor and non-tumor tissues of early-stage HBV-associated HCC found an increased expression of TGF-β genes compared with the non-tumor tissue[49]. However, the dichromatic role of TGF-β1 on cancer growth *i.e.,* tumor suppressive in early-stage or oncogenic effect in late-stage, is a matter of concern. Another study indicated a comparative functional genomic approach and illustrated the link between TGF-β expression signature and HCC subtypes. The study shows that TGF-β positive HCC clusters can be categorized into two. HCC with early TGF-β signature exhibit physiological responses while HCC associated with late TGF-β signature showed metastasis and poor survival[50]. N-2-fluorenylacetamide induced rat hepatoma model is used to investigate the association of TGF-β1 expression with different stages of hepatocarcinogenesis and a progressive elevation of hepatic TGF-β1 and TGF-β1 mRNA was found during the transformation of hepatocytes to malignant cells[51]. Another study indicates that elevated plasma TGF-β1 level was found in 89.5% of HCC patients, and interestingly, among these patients, 93.3% had an AFP level less than 400 µg/L[52]. This suggested that TGF-β1 expression can be a more accurate and sensitive biomarker for early diagnosis of HCC for monitoring the disease progression.

The diagnostic importance of TGF-β1 is established significantly earlier itself. In 1997, Tsai *et al*[53] investigated the correlation of urine TGF-β1 level with HCC. They found a significant increase in TGF-β1 level in HCC patients compared with other healthy control groups, cirrhotic chronic hepatitis patients. They also reported the association of TGF-β 1 levels with poor prognosis and shorter survival[53]. Later, the same team compared the study with another important tumor marker, α-fetoprotein, and found

that disease progression from cirrhosis to HCC is characterized by a typical elevation in both urinary TGF-β1 and serum AFP with a diagnostic accuracy of about 90%[54,55]. These collective data suggest the potential of TGF-β1 be used along with AFP as a complementary tumor marker to differentiate HCC from cirrhosis correctly. Another study investigated the rationale for parallel determination of TGF-β1 and AFP to diagnose HCC. They found that the TGF-β1 level exhibited a stage-dependent increase in all liver diseases where AFP showed HCC-specific elevation[56]. As TGF-β1 estimation tracks the disease progression pattern, TGF-β can be considered a more sensitive diagnostic marker of HCC. Its specificity is higher when it is analyzed along with AFP. To diagnose and select patients for galunisertib (TGF-β inhibitor) therapy, Cao Y *et al*[57] in 2017 performed next-generation sequencing-based analysis in HCC samples and found that m RNA levels of TGF-β1 along with SKIL and PMEPA1 could be better diagnostic markers as well as to select patients who are more likely to respond with galunisertib.

Another study investigated the association of serum TGF-β1 with disease severity in HCC using 180 subjects in different stages of HCC. Group of cirrhotic patients, as well as healthy control, was also maintained. Consistent with the previous reports, this study also found a significant increase in TGF-β1 level in HCC (1687.47 ± 1462.81 pg/mL) as compared with cirrhotic patients (487.98 ± 344.23 pg/mL) and control (250.16 ± 284.16 pg/mL). Additionally, the serum level of TGF-β1 showed exponential elevation as the disease progressed from early to advanced, *i.e.,* during progression from Barcelona Clinic Liver Cancer stage A to D, TGF-β 1 level increased from 652.83-1668.78 pg/mL[56]. The best cut-off value of TGF-β1 detection was determined as 301.9 pg/mL, comparable with the value (370 pg/mL) reported by Shehata *et al*[58] (Table 1).

Background inflammation and indolent transformation are the critical factors that create a waiting time paradox in diagnosing HCC, making the tumor more aggressive and refractory. Since TGF-β, specifically TGF-β1 plays an essential function from the initial hepatic injury to hepatocarcinogenesis, it holds immense potential to validate as a diagnostic marker of HCC. Though the dual functioning of TGF-β1 is still debatable, the

diagnostic relevance of TGF-β1 is well evident, and thus, it warrants further investigations and clinical validation.

The clinical utility of TGF-β1 as a prognostic marker of HCC

Poor prognostic characteristics of HCC contribute to late detection, aggressiveness and failure of therapeutic interventions[70]. Molecular pathways of hepato-carcinogenesis are still confusing because of the involvement of diverse molecular pathways, genetic alterations and evolution of malignant cells. Thus, this ultimately results in the worst prognosis within the early stage itself[71]. The expression of TGF-β1 is remarkably increased at the advanced stages of HCC and is involved in initiating EMT, regulating tumor proliferation, and promoting immunosuppressive tumor microenvironment during HCC progression under the challenges like liver cirrhosis, HBV and HCV infections. This warrants screening TGF-β1 levels from the early stages of HCC as a tool for evaluating the clinical outcomes. Depending upon the expression profile of TGF-β1, it is effortless to estimate the clinical impact of therapeutic strategies[72].

A research study conducted by Giannelli *et al*[73] proposed that TGF-β1 promotes EMT by stimulating homologous proteins like snail and slug. Secretion of TGF-β1 by HCC invasive cell lines, especially cell lines with α3β1-integrin expression, is significantly higher than in non-invasive and cirrhotic cell lines. The patients at the initial and advanced stages of HCC with a higher profile of TGF-β1 possess a poor prognostic ratio with lower overall survival (OS) and disease-free survival rate (DFS)[73,74]. Likewise, another notable experimental study by Lee *et al*[75] demonstrated that plasma TGF-β1 is positively correlated with critical conditions like EHM, portal vein thrombosis, EHM, and regional lymph node involvement. Statistical studies involving the detailed examination of overall and cumulative survival rates of HCC patients showed that candidates with abundant levels of plasma TGF-β1 manifested remarkably lower survival rates than the candidates with lower expression of TGF-β1. This evidence points to the usefulness of TGF-β1 as a prognostic marker in HCC.

Wang Y *et al*[76] elucidated the crucial involvement of TGF-β1 in tumor progression. A total of 180 patients with HCC were selected for the study, and out of 180 HCC patients, 105 patients were found with a solid expression of TGF-β1. This study showed a positive correlation between TGF-β1 and Treg cells. The increased secretion of TGF-β1 at the starting stage of HCC indicates that the tumor may be one of the most critical sources of TGF-β1 in HCC patients. Earlier studies also provided evidence that TGF-β1 promotes the regulatory phenotype and modulates the biological functions of Tregs. By Kaplan-Meier evaluation, HCC patients overexpressing TGF-β1 in neoplastic tissues had a considerably shorter OS and a greater recurrence rate than patients with lower expression[76]. A meta-analysis study conducted by Peng *et al*[77] reported that TGF-β1 implements an unfavorable prognosis on OS rates of HCC patients with a hazard ratio of about 1.71 and 2.29 from both univariate and multivariate analysis. Additionally, the study indicates the worst prognosis of TGF-β1 upon DFS, relapse-free survival and progression-free survival of 1422 patients *via* COX univariate analysis with a hazard ratio of about 1.60. In summary, the results from these studies draw out the negative prognostic impact of high TGF-β1 expression on the OS in HCC patients.

TGF-β1 possesses a dual functional role in malignancy; initially, it acts by blocking epidermal growth and promoting tumor suppression, but in later stages, it appears to be involved in the up-gradation of advanced tumors[78]. Embryonic liver fodrin (ELF), a novel form of β-spectrin is involved as a Smad3/4 adaptor in TGF-β mediated tumor suppression signaling pathway. Miscolocalization of Smad3 and Smad4 caused by the dysregulation of ELF resulted in the disruption of TGF- β signaling pathways[79]. A research study conducted by Ji *et al*[80] investigated the predictive value of both TGF-β1 and ELF in HCC patients after hepatic resection. The expression of TGF-β1 is significantly higher in HCC tissues than in normal liver tissues, while the incidence of ELF is higher in normal liver tissue in contrast with HCC samples. The reports of post-operative survival rates of HCC patients with lower expressions of TGF-β1 showed that DFS and OS rates of HCC patients over 1 (79.4%), 3 (73.5%), and 5 (62.0%) years were significantly higher than the patients with higher expression of TGF-β1 (28.0%, 12.0%,

and 12.0%). The study also showed a negative correlation between TGF-β1 and ELF levels. The study also indicates that DFS rates of HCC patients with higher expression of ELF and lower TGF-β1 Levels are remarkably more elevated than the HCC patients with low expression of ELF and higher TGF-β1 levels for 1, 3, and 5 years (75.0%, 60.0%, and 57.5% *vs* 25.0%, 15.9%, and 10.2%, respectively), with *P*-value less than 0.001. Data from clinicopathological examination exhibited that TGF-β1 positively relates with hepatitis B surface antigen, tumor size, tumor number, TNM, and recurrence, while ELF is negatively correlated with all metastatic characteristics suggesting that ELF is associated with tumor suppressing features. This research study indicates that both TGF-β1and ELF can be included in the category of relevant biomarkers as prognostic agents for evaluating clinical results after hepatic resection[80].

An experimental approach described the correlation and the possibility of Fibroblast growth factor (FGF) receptor 4 (FGFR4) and TGF-β1 as prognostic biomarkers in HCC. FGFR4 is the most predominant isoform of the FGF receptors family and is actively involved in various biological activities, including metastasis, differentiation, embryonic development, proliferation, apoptosis and angiogenesis[81]. Multiple studies showed that FGFR4 plays a clear-cut role in the pathogenesis of HCC and the up-regulation of FGFR4 possesses resistance to various targeted therapies[82]. A clinicopathological examination conducted by Chen *et al*[83] showed that elevated expression of both TGF-β1 and FGFR4 enhances tumors' invasiveness and metastatic nature. Clinicopathologic characteristics revealed that HCC patients at advanced stages with high TGF-β1 and FGFR4 expression were more likely to be at a higher TNM stage. Statistical data showed that the OS of patients over five-year survival rate is about 8.5%, and the median survival duration is 32.3 mo in case of TGF-β1 positive cases. In contrast, in TGF-β1 negative expression cases, the OS of the patient is about 45.6% and the median survival rate is 50.4 mo. Candidates with high TGF-β1 expression had a short OS rate in contrast to those with negative TGF-β1 expression profiles. Likewise in cases of high levels of FGFR4, the OS rate of patients is very low, that is five-year survival rate is only about 8.3%, and median survival rate is 29.4 mo while in the

condition of impeded FGFR4 expression, the five year survival rate is about 70.1% and the median survival period is 51.2 mo. This study showed a positive correlation between TGF-β1 and FGFR4 as prognostic markers in HCC. Additionally, the results from univariate and multivariate analyses showed that both TGF-β1 and FGFR4 are independent and reliable prognostic factors in HCCs for evaluating the therapeutic response in HCC patients, especially after post-operative procedures[83]. The strong correlation between TGF-β1 expression and survival rates of HCC patients suggests its potential as a prognostic biomarker for HCC (Table 2). In addition to the role of TGF-β1 as an effective prognostic marker, it can also be used for targeted therapeutic strategies.

The clinical utility of TGF-β1 as therapeutic target in HCC

Tyrosine kinase inhibitor, Sorafenib was the first approved first-line therapy for advanced HCC. Sorafenib was the first-line therapy for ten years until another tyrosine kinase inhibitor, levatinib was approved in 2018. Even though tyrosine kinase inhibitors dominated HCC therapy as first-line or second-line options, the efficacy was only modest, with limited treatment outcome, the emergence of drug resistance, and also relapse[85]. Recently treatment strategies adopted a paradigm shift to immunotherapeutic approaches because of the importance of the immune microenvironment in carcinogenesis.

The liver is the most extensive reticuloendothelial system and peripheral immunomodulatory organ in the human body. Immunotherapy is being extensively evaluated in liver cancer[86]. The liver constitutes a vast repository of immune cells including NK cells, kupffer cells, sinusoidal endothelial cells, and innate T cells[87]. Aberrant immune checkpoint activation makes HCC a cold tumor, where anti-tumor immune surveillance is completely abolished[88]. Combination of immune checkpoint inhibitors (ICIs) such as Nivolumab (PD-1 inhibitor) + Ipilimumab (CTLA4 inhibitor) and Atezolizumab (PD-L1 inhibitor) + Bevacizumab [vascular endothelial growth factor (VEGF) inhibitor] got approval as first-line therapy for various cancer such as non-small cell lung cancer (NSCLC), HCC in 2020[89]. Though ICIs exerted a superior effect to

tyrosine inhibitors, current immunotherapeutic drugs failed to establish an effective anticancer immunity against HCC. Though the immunotherapeutic approaches modify effector immune cells functions to elicit anti-tumor immune response, the immunosuppressive tumor microenvironment neutralizes the effects of immunotherapy[90].

Analysis of the TGF-β profile of HCC samples in the TGCA data set revealed four categories with typical TGF-β expression[91]. The cluster with a highly activated TGF-β signature, which accounts for 14.5% of samples, exhibited prominent immune exhaustion and poor prognosis. ICIs may not work well in this cluster. Additionally, anti-inflammatory/anti-fibrotic agents targeting TGF-β can improve the immune milieu[92,93]. The majority of HCC samples belong to a cluster of activated TGF-β signature (45%) and showed a low level of an immune response. Hence, combining a TGF-β inhibitor with an immune checkpoint inhibitor can exert a synergistic effect. 30% of HCC samples showed a normal TGF-β signature associated with active immune surveillance; therefore, immunotherapy will be most suitable for this cluster. The fourth cluster is a minor population (9.9%) that exhibits inactivated TGF-β signature with poor immune cell activation and response[94]. With this evidence, it is clear that TGF-β1 signature can be used to decide the suitable therapy or predict the outcome of immunotherapy.

Several specific and non-specific inhibitors of TGF-β, mainly TGF-β1 and 2 inhibitors, are being developed and evaluated against various tumors, including HCC. Regarding non-specific inhibitors, as the primary molecular target is different, its ability to block the TGF-β pathway offers additional benefits as anticancer agents. One such example is Halofuginone, an alkaloid coccidiostat with reported preclinical activity against HCC. In addition to the prominent inhibition of collagen synthesis, Halofuginone also blocks TGF-β1, inhibits ECM formation and fibroblast proliferation, increases IFN-γ and anti-tumor immune response[95,96]. The effect of Halofuginone against advanced progressive solid tumor has been evaluated in phase I clinical trial (NCT00027677), and in 2000, United States Food and Drug Administration gave orphan drug approval status to

Halofuginone for treatment of scleroderma[97]. Histone deacetylase exerts epigenetic regulation of TGF-β1 mediated fibrosis and carcinogenesis[98]. Studies indicate that histone deacetylase inhibitors such as Panobinostat have shown effectiveness in HCC animal models and phase I human trials combined with sorafenib (NCT00823290)[99]. Apart from the non-specific inhibitors, recent preclinical interventions combined TGF-β1 targeting antibodies or TGF-βR1 inhibitor with PD-L1 inhibitors and obtained prominent cytotoxic effect and anticancer immune surveillance in various solid tumors[100,101]. Likewise, M7824 is a bifunctional fusion protein with dual targeting of PD-L1 and TGF-β, which has been evaluated in animal models of various cancers either alone or in combination with vaccines[102]. M7824 exerted a significant inhibitory effect on TGF-β1[103]. Among the different TGF-β inhibitors, LY2109761 is an orally bioavailable TGF-β receptor type I inhibitor, which exhibited an antitumor effect in various HCC animal models[104]. LY2109761 inhibited TGF-β1 induced migration, invasion, and anoikis in HCC cells[105,106]. Another study suggested the anti-angiogenic potential of LY2109761, which was superior to the typical VEGF inhibitor, bevacizumab, and the effect was mediated by suppression of VEGF through inhibition of SMAD dependent TGF-β 1 signaling[107,108]. In addition to this, anti-TGF-β agents targeting other isoforms are also developed. AP-12009 is a TGF-β2 specific antisense oligonucleotide in a clinical trial to treat glioma and anaplastic astrocytoma[109,110]. Likewise, TGF-β1 directed mRNA was developed as AP-11011, which is evaluated against NSCLC, colon cancer in preclinical models[111]. Lordelimumab is a TGF-β2 specific monoclonal antibody with an anti-fibrotic effect[112]. Many preclinical studies investigated different TGF-β inhibitors, yet, galunisertib (LY2157299), a kinase inhibitor of TGF-β1, is only the one in current clinical trials[113]. Several clinical trials of galunisertib are ongoing or completed either alone or combined with sorafenib, ICIs, and alkylating agents (Table 3).

CONCLUSION

TGF-β1 exerts a unique regulatory power on inflammation, fibrogenesis, and immune response in HCC. Among other TGF-β isoforms, significant and progressive expression of TGF-β1 during the entire course of HCC pathogenesis, starting from chronic hepatitis to HCC makes it a sensitive and accurate diagnostic marker of HCC. The specificity and sensitivity of TGF-β1 based diagnosis of HCC by parallel estimation of serum AFP. Even after establishing HCC, TGF-β1 continues to elevate as HCC progresses and is associated with poor prognosis and shorter survival. Since TGF-β1 is the master regulator of the immunosuppressive tumor milieu in HCC, TGF-β1 inhibition could sensitize ICI, tyrosine kinase inhibitors, and other systemic or curative interventions. HCC remains the deadliest-refractory tumor predominantly due to its delayed diagnosis. In that context, TGF-β1 is relevant for early diagnosis, prognosis, and therapy. Even though a plethora of supporting evidence is available, still TGF-β1 is not much studied and evaluated compared with other markers such as AFP. Notably, the dichotomic nature of TGF-β signaling in HCC needs to be defined accurately to establish the clinical utility of TGF-β1. Thus, proper and careful determination of the TGF-β1 profile of patients is necessary to choose the suitable patients for TGF-β1 targeted therapy.

76266_Auto_Edited-check.docx

ORIGINALITY REPORT

3%
SIMILARITY INDEX

PRIMARY SOURCES

1	Yi Wang, Taotao Liu, Wenqing Tang, Bin Deng, Yanjie Chen, Jimin Zhu, Xizhong Shen. "Hepatocellular Carcinoma Cells Induce Regulatory T Cells and Lead to Poor Prognosis via Production of Transforming Growth Factor-β1", Cellular Physiology and Biochemistry, 2016 Crossref	36 words — 1%
2	link.springer.com Internet	33 words — 1%
3	Haiyan Zhu, Hui Luo, Zhaojun Shen, Xiaoli Hu, Luzhe Sun, Xueqiong Zhu. "Transforming growth factor-β1 in carcinogenesis, progression, and therapy in cervical cancer", Tumor Biology, 2016 Crossref	22 words — < 1%
4	www.researchsquare.com Internet	18 words — < 1%
5	M. Artuc. "Mast cells and their mediators in cutaneous wound healing ? active participants or innocent bystanders?", Experimental Dermatology, 2/1999 Crossref	15 words — < 1%
6	www.scilit.net Internet	13 words — < 1%

7	Mohamed Ahmed Samy Kohla, Ahmed Attia, Nehad Darwesh, Manar Obada, Hossam Taha, Marwa F. Youssef. "Association of serum levels of transforming growth factor b1 with disease severity in patients with hepatocellular carcinoma", Hepatoma Research, 2017 Crossref	12 words — < 1%
8	testmobile.mims.com Internet	12 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 12 WORDS
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS