The TGF-β Signaling Pathway as a Pharmacological Target in Hepatocellular Carcinoma

Antonio Mazzocca, Salvatore Antonaci and Gianluigi Giannelli*

1. INTRODUCTION

Research into new mechanism-based drug therapies for the treatment of patients with hepatocellular carcinoma (HCC) has become more competitive since sorafenib was introduced for the treatment for HCC patients with advanced disease [3]. As in other malignancies, the issue is to identify suitable pharmacological molecular targets and agents that exert little or no cytotoxicity. In HCC, this is particularly desirable in view of the presence of the liver cirrhosis which is commonly associated to HCC and limits the use of many drugs. Even the use of a multi-target inhibitor, like sorafenib, that fights the cancer via inhibiting several signaling pathways, can be limited by numerous side effects. In fact, this type of drug should, in theory, display more side effect compared to drugs directed against only a single target. Tailoring the therapy to the individual patient would be a promising strategy to circumvent this problem [4]. For this reason, many efforts have been made to achieve molecular characterization of those patients who are eligible for treatment with a certain drug. In theory, this could be achieved by identifying the specific gene signature, or even better a kinases signature (i.e. a profile of activity of protein kinases involved in response to the drug). An alternative strategy would be to drive preclinical research faster in the direction of identifying the mechanisms responsible for HCC development and progression. This could lead to the identification of key molecules actively participating in the process of tumor progression, that could then be targeted by appropriate drugs [5]. Clearly, to achieve results that can be translated to humans, such preclinical research should provide reliable in vivo models of HCC which replicate, at least in part, the tumorigenic phases occurring in humans. Although animal models of liver cirrhosis and hepatitis viruses are still lacking, a wider range of animal models is now available to study HCC [6,7]. These animal models are becoming useful tools for pharmacological studies in HCC and will hopefully improve the preclinical findings to be translated to clinical studies.

2. PATHOGENESIS OF HCC

Fibrotic liver disease and chronic inflammation often precede the onset of HCC by about 10 or 20 years, which suggests that these may act as a precancerous condition for HCC. Therefore, HCC can be seen as a multi-step carcinogenetic process [2]. The role of chronic viral hepatitis (HBV and HCV) in HCC is well-established [8]. These pathological conditions are characterized by varying degrees of fibrogenesis, considered as a predisposing factor for the onset of HCC [9]. As in other tissues, fibrosis of the liver is considered to be partly a physiological response triggered to repair liver damage. However, when the liver fibrosis becomes chronic, the repair mechanisms and inflammation associated with this chronic situation can become irreversible and lead to the condition of cirrhosis. Some pathological aspects of liver cirrhosis are very important, such as nodular hyperplasia, which is considered a premalignant lesion from which HCC develops [10]. Typically, HCC is a multinodular disease even when it is first detected as a single node. The majority of HCC forms are well-circumscribed nodular tumors, round or oval, without a capsule. Four distinct microscopic patterns have been identified: (1) fibrolamellar; (2) pseudoglandular (3) pleomorphic/giant cell; (4) clear cell. HCC presents more differentiated hepatocyte trabeculae, cords and nests, and contains pigments or bile, while poorly differentiated tumors show malignant cells that are not cohesive, pleomorphic, or anaplastic giant cells. Because of these important features, the histology and tumor staging will take into account the size and differentiation of the lesions, and will include the assessment of serum α-fetoprotein, the spread (for example, the presence of vascular invasion and the presence of intra- and extrahepatic metastases) and the degree of tumor vascularization (angiogenesis). These aspects are important to estimate the expected survival and determine the type of intervention. In an attempt to combine the clinical and laboratory predictors of outcome in patients with HCC, several staging systems have recently been proposed, including Barcelona Clinic Liver Cancer staging (BCLC) that is currently the most widely approved and recommended for use in clinical trials [11,12].

3. ROLE OF TGF-β1 IN TUMOR SUPPRESSION AND CANCER PROGRESSION

Clinical data suggest that TGF-β1 may play an important role in HCC progression. This is not the only consideration that could jus-
4. ROLE OF TGF-β IN FIBROGENIC LIVER DISEASES AND HCC

The role of TGF-β in liver fibrogenic disease and HCC is well-documented. The TGF-β signaling pathway consists of three distinct ligands, TGF-β1, TGF-β2, and TGF-β3. All three ligands can bind to a specific receptor, firstly engaging with TGF-βRI, which then heterodimerizes with TGF-βRII. This heterodimer complex phosphorylates the intracellular Smad2 and 3 proteins, which in turn gives rise to a cascade of activation to induce different nuclear proteins transduction [32]. TGF-β has been investigated because of its association with organ fibrosis and collagen synthesis, where it is evaluated as a potential marker [33,34]. In fact, TGF-β1 levels are elevated in fibrosis associated with both viral and alcoholic hepatitis. Moreover, in patients with documented HBV serum levels, TGF-β1 levels are associated with fibrosis, and in HCV patients who respond to treatment with interferon-alpha and ribavirin, the TGF-β1 levels decrease [35,36,37].

Liver fibrosis consists of an accumulation of stiff, fibrous scar tissue in the liver. This process is associated to chronic viral or alcoholic hepatitis and becomes more prominent in liver cirrhosis. Hepatic stellate cells (HSC) are specialized pericytes characterized by their ability to store retinol, that play a central role in liver fibrogenesis [38] [39]. After acute or chronic injury affecting hepatocytes, HSC are activated and transdifferentiate into myofibroblasts [40]. As a consequence, these cells activate an autocrine or paracrine TGF-β loop. Upon TGF-β stimulation, HSC produce extracellular matrix (ECM), and in particular proteins such as fibronectin and collagen [41] [42]. Moreover, the secretion of TGF-β creates a response by the immune system that prevents the down-regulation of the inflammatory process in the liver [43] [44] [45]. Gene expression and functional studies show that in patients with chronic liver fibrosis, liver tissue with a rich TGF-β content contains a large number of regulatory T cells [47,43,44].

As pointed out above, TGF-β has a dual role, acting as a tumor suppressor that regulates cell proliferation, but also as a tumor promoter facilitating the motility and invasion of cancer [48]. This dual effect is well known but is still controversial, and how and why TGF-β acts differently in the different stages of cancer progression is still unknown. One of the first studies on TGF-β1 levels in HCC was performed in six patients who showed an increased TGF-β expression at mRNA and protein level [49]. A few years later, the same authors were able to correlate the tissue expression with increased plasma levels of TGF-β1 in patients with HCC, and these levels have been shown to be higher than in patients with chronic hepatitis and cirrhosis [50]. In addition, the levels of TGF-β1 decreased in patients treated for liver cancer, suggesting its potential use as a marker for monitoring the response of patients to treatment. In addition to plasma levels, urinary TGF-β1 levels were also found to have prognostic value, resulting in poor survival in patients with HCC [51]. Moreover, high levels of TGF-β1 were found in patients with aggressive and functional studies show that in non-metastatic HCC [52]. Because a high vascular density is linked to poor survival, it is not surprising that high tumor vascular density is associated with high levels of TGF-β1, and then correlated with aggressive tumor growth [53] [54]. Patients with vascular invasion display worst prognosis and shorter survival [55,56,57,58]. Measurement of TGF-β1 levels in plasma of patients with HCC is feasible and may have a prognostic value [59] [51]. To increase the prognostic value of TGF-β1 levels, it is possible to combine the levels of TGF-β1 with other protein markers. When TGF-β1 is combined with α-fetoprotein (AFP) it seems to improve the diagnostic predictability [60,61]. However, the difficulty of obtaining a reproducible prognostic marker is not only linked to the heterogeneous nature of the cancer, but also to the fact that sample recovery and analysis must be performed with great care and precision, to avoid confusing the liver-derived TGF-β with the platelet-derived TGF-β isoform, for example. As in liver fibrosis, TGF-β levels increase in HCC, along with collagen deposition and the reduction of proteolytic degradation [62]. Another factor with stromogenic properties, modulated by TGF-β in the liver, is connective tissue growth factor (CTGF) [63]. CTGF promotes tumor growth, angiogenesis, migration and invasion [64] [25]. HCC human cell lines produce high levels of CTGF to form highly stromogenic tumors. If CTGF is knocked down in these cells, tumors show a poor stromal component [26]. Blocking the TGF-β signaling pathway with the TGF-β inhibitor LY2109761 inhibits CTGF production and tumor growth. Based on these observations, cancer-associated fibroblasts (CAF) are considered a possible source of CTGF in response to paracrine signals from tumor cells, such as TGF-β [64,65,66].

5. INHIBITION OF TGF-β SIGNALING IN HCC

Pharmacological inhibition of TGF-β1 has recently been proposed by different authors as an anti-cancer therapeutic approach [67,68] [69]. Clin Cancer Res 2007; [70]. We have proposed that pharmacological inhibition of TGFβR can be considered a new
Fig. (1). Schematic representation of the multifunctional contribution of TGF-β1 in HCC progression. TGF-β1 intervenes in various biological steps during HCC progression. It is involved in regulation of the tumor-stroma cross-talk, neo-angiogenesis, intravasation and cancer dissemination. Because of its involvement in multiple steps during tumor progression, blocking the TGF-β1 signaling axis with TGFβR inhibitors could prove to be a promising pharmacological approach to target different steps of HCC progression.
paradigm that by “hitting one target leads to a domino effect”. This is easy to understand since the inhibition of a pleiotropic factor like TGF-β1 indisputably leads to the inhibition of several downstream effectors. The concept of a pharmacological inhibition of TGF-β1 is different from that of a multikinase inhibitor. The former is based on the fact that blockade of one single target leads to the inhibition of multiple effectors, while the latter involves the inhibition of different kinases to elicit a similar effect [71]. The advantage of “the domino effect” is that it has a more efficient mechanism of action, better pharmacodynamics and a lower toxicity. LY2109761 is a selective inhibitor of the TGF-β1 receptor kinase belonging to a new class of compounds now used to inhibit TGFβR. The chemical structure of LY2109761 and other compounds belonging to different classes of TGF-β1 receptor kinase inhibitors are shown in (Fig. 2). LY2109761 was discovered during screening to test the structure-activity relationships (SAR) of the quinoline domain of dihydropyrrolopyrazoles [72]. This drug selectively blocks the activation of SMAD2, a marker of TGFβR activation, at concentrations ranging from 0.001 to 0.1 microMolars. Moreover, LY2109761 inhibits migration of HCC cells on fibronectin, laminin-5, and vitronectin, as well as tumor cell invasion through Matrigel at concentrations up to 0.1 microMolar, and up-regulates expression levels of E-cadherin in nonmetastatic HCC tissues treated ex-vivo [73]. LY2109761 has shown an interesting anti-tumor activity against both solid and haematological malignancies. For example, It suppresses pancreatic cancer metastasis to the liver and, in combination with gentacytabin, reduces the tumor mass and increases the survival rate of treated animals [74]. Another study, conducted by Xu et al., showed that LY2109761 inhibits TGF-β1 produced by bone marrow stromal cells that sustain the survival and chemotherapy resistance of leukaemia cells [75]. Recent studies have shown that LY2109761 decreases metastases to the liver and prolongs survival of mice in a murine syngeneic model of colon cancer [76,77]. Another study investigating the capacity of HCC cells to invade the blood vessels, that is one of the major negative prognostic factors in patients with HCC, has shown the impact of inhibition of TGFβR by LY2109761 on this process [78]. In particular, this study showed that invasion of blood vessels is mediated by TGFβR, that transactivates β1 integrin via SMAD2 and SMAD3. TGF-β1 induces the phosphorylation of the intracytoplasmic tail of β1 integrin on threonine 788-789 through an “inside-out” mechanism of activation. Because the inhibition of TGFβR by LY2109761 blocks vascular invasion of HCC cells, this may suggest a rationale for targeting the TGF-β1 signaling pathway to inhibit the metastatic dissemination of cancer cells. The antitumor activity of LY2109761 is also associated with the inhibition of molecular pathways involved in neo-angiogenesis and tumor growth of HCC [79]. LY2109761 has shown a promising anti-angiogenic effect that seems to be more effective than the one exerted by bevacicuzmab, a well-established anti-vascular endothelial growth factor (VEGF) monoclonal antibody. LY210976 blocks the paracrine cross-talk between HCC and endothelial cells and hence the formation of new blood vessels [79]. This effect is mediated by the SMAD2/3 signaling pathway which regulates the secretion of VEGF. Interestingly, LY2109761 has not shown any significant effect on physiological angiogenic development. This study supports the idea that targeting TGF-β signaling in patients with HCC could eventually affect neo-angiogenesis. The take-home message from all these studies is that pharmacological targeting of TGF-β signaling pathway may result in simultaneously blocking several downstream effectors, cellular functions and different processes underlying tumor progression. Blocking one single target, such as TGFβR, which in turn results in inhibition of several downstream functions, is a novel concept. Hitting one single target that leads to a domino effect is an attractive and alternative pharmaceutical concept, but it is not mutually exclusive to that of multitarget/kinase inhibition, in which sorafenib is a paradigm in the treatment of HCC. Therefore, it is plausible that a combination of drugs acting with different mechanisms (i.e. TGFβR inhibitors in association with multikinase inhibitors) may be a promising strategy for the treatment of HCC in the near future.

6. FUTURE PERSPECTIVES

Medical therapy of HCC is an intriguing but not an easy issue, since the pharmacological approach has to take into account the liver cirrhosis that so often underlies human HCC. So far, sorafenib is the sole pharmacological therapy that has revealed some efficiency in terms of improved survival in HCC patients. Sorafenib is an oral multikinase inhibitor that acts on several signaling pathway including the Raf/MEK/ERK pathway, VEGFR and PDGFR [80]. Sorafenib is currently the gold standard therapy to which new anti-HCC drugs must be compared. In addition, new target-based therapy needs to be tailored to the individual patient and therefore the molecular target has to be tested before starting the treatment. This means that if the molecular target is TGFβR, this must be expressed and validated in terms of the specific biological activity in the patient who is a candidate to receive TGFβR inhibitors. This is currently the main challenge that LY2109761 or other TGFβR inhibitors have to overcome to successfully enter the arena of human therapeutic approaches. The availability of a reliable and selective inhibitor is not enough to treat cancer in patients if detailed information on the expression and function of the target is lacking. As
described in this review article, preclinical studies of TGFBR inhibitors (i.e. LY2109761) have recently been conducted. However, more accurate investigations on the drug mechanisms, pharmacokinetics and pharmacodynamics are needed to better evaluate whether this class of inhibitors can rapidly enter early clinical development in HCC.

7. CONCLUDING REMARKS

Despite the dual role of TGF-beta in cancer, the use of TGFBR inhibitors is proving to be a promising anti-cancer approach in preclinical studies. However, additional studies are needed to confirm the efficacy of this class of inhibitors and to evaluate their toxicity. In HCC, the powerful rationale for evaluating TGF-beta lies in the fact that TGF-beta is involved in tumor progression and regulates several steps related to the interactions with the microenvironment, including the intravasation of tumor cells, angiogenesis and the production of extracellular matrix. In addition, TGF-beta promotes liver fibrosis and its inhibition could turn out to be beneficial for HCC patients with underlying liver cirrhosis. While the scientific debate as to whether TGF-beta acts as a promoter or suppressor of oncogenesis is still ongoing, a growing body of evidence suggests that inhibition of TGF-beta eventually exerts anti-tumor activities. However, these findings must be treated with some caution, and the preliminary data we have obtained so far on TGF-beta inhibitors need to be validated in a broader number of studies and in different preclinical cancer models before setting up clinical trials. Finally, the ongoing development of new classes of TGF-beta inhibitors should improve our arsenal of anti-cancer drugs, as well as promoting a better understanding of the biology of TGF-beta in the cancer process.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

The Authors are grateful to Mary V. Pragnell, B.A., for language revision.

ABBREVIATIONS

HCC = Hepatocellular Carcinoma
TGF-beta = Transforming growth factor-beta
TGFBR = Transforming growth factor-beta receptor
AFP = Alpha-fetoprotein
CTGF = Connective tissue growth factor
CAF = Cancer associated fibroblasts
SAR = Structure-activity relationships
SMAD2/3 = Small mother against decapentaplegic
VEGF = Vascular endothelial growth factor
MEK = Mitogen-activated protein kinase kinase
ERK = Extracellular signal-regulated kinase
PDGFR = Platelet-derived growth factor

REFERENCES


Kawada N. The hepatic perisinusoidal stellate cell Histol Histopathol 1997; 12: 1069-80


Winu F, Quack C, Darmoise A, Kaufmann SH. Starring stellate cells in liver immunology Curr Opin Immunol 2008; 20: 68-74


Giannelli G, Antonaci S. Immunological and molecular aspects of liver fibrosis in chronic hepatitis C virus infection Histol Histopathol 2005; 20: 939-44


Leak A, Abraham DJ. All in the CCN family: essential matricellular signaling modulators emerge from the bunker J Cell Sci 2006; 119: 4803-10


