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TGF- β Signaling in Liver and Gastrointestinal Cancers

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Abstract

Transforming Growth Factor- β (TGF- β) plays crucial and complex roles in liver and gastrointestinal cancers. These include a multitude of distinct functions, such as maintaining stem cell homeostasis, promoting fibrosis, immune modulating, as a tumor suppressor and paradoxically, as a tumor progressor. However, key mechanisms for the switches responsible for these distinct actions are poorly understood, and remain a challenge. The Cancer Genome Atlas (TCGA) analyses and genetically engineered mouse models now provide an integrated approach to dissect these multifaceted and context-dependent driving roles of the TGF- β pathway. In this review, we will discuss the molecular mechanisms of TGF- β signaling, focusing on colorectal, gastric, pancreatic, and liver cancers. Novel drugs targeting the TGF- β pathway have been developed over the last decade, and some have proven effective in clinical trials. A better

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Conflict of interest

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understanding of the TGF- β pathway may improve our ability to target it, thus providing more tools to the armamentarium against these deadly cancers.

Keywords

TGF- β Signaling; cancers; liver cancer; GI cancers

Introduction

The gastrointestinal (GI) tract is responsible for more cancers and more cancer deaths than any other organ system in the body [1]. Colorectal cancer (CRC) is the third most common cancer in the Western world, comprising 9% of the cancer burden in both genders. Pancreatic cancer is responsible for 3% of cancer cases. The incidence of hepatocellular carcinoma (HCC) in the U.S. has tripled over the last two decades [2]. Gastric cancer remains the second leading cause of cancer mortality. Pancreatic, biliary, and HCC share a poor prognosis and usually are diagnosed at advanced stages. CRC mortality rates are declining in several Western countries [1], mainly because of increased awareness, early detection, and improved treatment [3]. However, late-stage CRC continues to have a dismal survival rate, with over 45% of patients dying of recurrence despite adjuvant therapy, with over 130,000 new cases diagnosed in 2016 in the United States [4]. Overall, there is an urgent need for the development of new drugs to treat these cancers in advanced stages. The Transforming Growth Factor- β (TGF- β) pathway is an important regulator of intestinal homeostasis and plays a major role in modulating GI cancers [5-7]. In the gastrointestinal tract, TGF- β is known to function as a promoter of fibrosis, immune modulator, tumor suppressor, and also as a tumor promoter. In this review, we will discuss the molecular mechanisms of TGF- β signaling and its role in liver and gastrointestinal cancers. Some of the newly developed drugs opposing TGF- β will be discussed as well.

Molecular Mechanisms of TGF- β Signaling

The TGF- β family contains about 40 structurally related factors that comprise TGF- β s, activins, inhibins, bone morphogenetic proteins (BMPs) and growth and differentiation factors (GDFs) [8] [9]. Three TGF- β isoforms are expressed in mammals (TGF- β 1, TGF- β 2, TGF- β 3). TGF- β 1 is the most abundant and well-studied isoform. The bioactive ligands are composed of homo- or hetero-dimers of polypeptides, synthesized as precursor molecules and matured by proteolytic cleavage by endoproteases. Active TGF- β dimers mediate signaling through the TGF- β type I and type II receptors (T β RI and T β RII, respectively), which are active serine/threonine kinases. A heterotetramer of two T β RI and two T β RII molecules comprises the functional receptor [9, 10]. The membrane-anchored β glycan, TGF- β type III receptor, assists TGF- β binding to T β RII [11]. Due to its dimeric structure, TGF- β is able to interact simultaneously with both type I and type II receptors. Binding the ligand to the extracellular domain of T β RII triggers cross-phosphorylation of T β RI by T β RII, activating its kinase activity, which then propagates the signal transduction through phosphorylation of the Smad proteins [12]. The Smad proteins are divided into three classes: receptor-regulated Smads (R-Smad), the common mediator Smad (co-Smad), and the

inhibitory Smads (I-Smad). R-Smads include Smad1, Smad2, Smad3, Smad5 and Smad8. R-Smads act as direct substrates of specific type I receptors. While Smad1, Smad5, and Smad8 are targets of BMP receptors, Smad2 and Smad3 are substrates of TGF- β receptors [13-15]. Once phosphorylated, R-Smads associate with the common Smad, Smad4, and mediate nuclear translocation of the heteromeric complex [16]. In the nucleus, Smad complexes then activate specific genes such as integrins, E-cadherin, through cooperative interactions with DNA and other DNA-binding proteins FAST1, FAST2, and Fos/Jun, ATF2, TFE3, PEBP2/CBF, Vitamin D receptor [17-20]. Transcriptional activation requires R-Smads (except Smad2) and Smad4 binding to DNA sequences despite the fact that these have a 100-fold lower affinity than the interacting high affinity DNA binding transcription factors [21]. TGF- β -induction of the PAI-1 and human collagenase promoters involves transcriptional regulators such as AP1 [22, 23], whereas TGF- β mediated transactivation of cyclin-dependent kinase inhibitors p21 [24] and p15 involves SP1 [25]. Another tumor suppressor co-factor is the transcription factor Runx3, whose expression is lost during gastric carcinogenesis, leading to resistance to growth inhibitory responses by TGF- β [26]. Runx3 cooperates with Smads during p21 transcriptional induction in stomach epithelial cells, thus providing a tissue-specific mechanism of the cytostatic, p21-mediated response [27]. The tumor suppressor protein tuberous sclerosis complex 2 (TSC2) is an established downstream effector of signaling by the LKB1 tumor suppressor [28]. TSC2 mediates the anti-proliferative effects of LKB1 in epithelial cells and represents an interesting nodal point of convergence between two major tumor suppressor pathways, TGF- β and LKB1. E3 ligases that include SMURF1, PRAJA, adaptor proteins such as β 2SP, SARA, nuclear phosphatases such as PPM1A control transcriptional activation of R-Smad/Co-Smad [5, 7, 29-33]. Smad6 and Smad7 are considered to inhibit ligand-dependent signaling [34, 35]. Smad6 binds to receptor activated Smad1, preventing Smad1 association with Smad4. Smad7 induces Smurf inactivation of TGF- β and BMP receptors, with an allelic association in colorectal cancers [36-38]

TGF- β signaling in stem cells and cancer: mouse models and human mutations

Mouse knockout studies combined with human mutations have provided important insight into gut epithelial and stem cell biology, and even more so to the pathways involved in gastrointestinal tumorigenesis. For instance, knockouts have revealed that Smad4 is required for gut endoderm lineage; Smad2 is needed for gastrulation and Smad3 for establishment of the GI mucosal immune response to TGF- β signals [39]. *Smad3*^{-/-} deficient mice frequently develop gut abscesses and die between 1 and 10 months due to impaired mucosal immunity. Chronic intestinal inflammation is infrequently associated with colonic adenocarcinoma in *Smad3*^{-/-} mutant mice older than 6 months of age. Defective liver development with loss of GI epithelial cell shape and polarity occurs in *β 2sp*^{-/-} homozygotes, *Smad2*^{+/-}/*Smad3*^{+/-} double heterozygotes, and strikingly, HCC in *β 2sp*^{+/-} heterozygotes [30, 40].

A recent finding has been the nearly identical phenotype of *β 2sp*^{+/-}, *β 2sp*^{+/-}/*Smad3*^{+/-} mice to a human hereditary cancer stem cell syndrome, the Beckwith-Wiedemann syndrome (BWS), associated with loss of imprinting of Insulin like growth factor 2 (IGF2) with

aberrant regulation of IGF2 by a chromosomal networking protein CCCTC binding factor (CTCF), the only known factor with chromatin insulator function in vertebrates. Increased activation of IGF2 with loss of CTCF in the $\beta 2SP^{+/-}$ and $\beta 2SP^{+/-}/Smad3^{+/-}$ HCCs was observed in these mutants [41]. Both mouse mutants and human patients with BWS develop multiple types of liver cancers as well as pancreatic and colon cancers. Further analyses of the effects of TGF- β signaling on tumor-initiating stem-like cells (TICs) revealed that knockdown of $\beta 2SP$ promotes Nanog-mediated CD133+/CD49+ tumor-initiating-cell (TIC) tumorigenesis in alcohol-fed HCV Ns5a Tg mice. Nanog expression as well as markers for cell proliferation in tumor initiating cells (TICs) that are stem-like is significantly increased with knockdown of $\beta 2SP$ [41]. In vivo, knockdown of Smad3 in the TIC cells increased tumor growth [41]. Further individual knockdown of each element of the complex (i.e. $\beta 2SP$, Smad3, or CTCF) in HepG2 cells results in an increase in ALDH+ (a stem cell marker) cell populations as well as increased sphere formation, which are two additional hallmarks of stemness. Interestingly, ALDH1A1, expression is increased in cell lines derived from BWS patients. The increase in ALDH+ cell populations following knockdown of $\beta 2SP$, Smad3, or CTCF restores the stemness phenotype of BWS cells. Thus the CTCF/ $\beta 2SP$ /Smad3 complex is involved in some aspects of stem cell biology in both mouse and human liver and gastrointestinal tissues [41]. $\beta 2SP$ and Smad3 co-occur within the same tumors- in pancreatic adenocarcinoma, kidney chromophobe, nasopharyngeal carcinoma, head/neck squamous cell carcinoma, and uterine corpus endometrial carcinoma [42, 43]. However, mutations in the TGF- β pathway are only part of the repertoire of loss of function. For instance, other mechanisms, such as epigenetic regulation of the TGF- β pathway, context-dependent responses and regulation of the TGF- β pathway generate cell, tissue, and cancer specificity can lead to mechanistic loss of function in human disease reflecting the knockout phenotype [41, 44, 45]. Moreover, while gene dosage is critically important in this family, as haploinsufficiency phenotypes result in aberrant gut, brain and liver hypoplasia as well as GI carcinoma, other mechanisms such as targeted protein proteolysis also play an important role in regulating protein levels [46].

TGF- β as a Tumor Suppressor and Immune Modulator (Figure 1)

TGF- β is a prominent anti-proliferative agent with a strong cytostatic effect. It inhibits cell cycle progression through G1 arrest. This is done both by inducing CDK inhibitors p21^{Cip1} and p15^{Ink4b}, and by suppressing proliferative drivers such as c-Myc and ID [9, 47]. Unlike the TGF- β cytostatic program, there is not a unique TGF- β -induced apoptotic program. Instead there are Smad-dependent and Smad-independent mechanisms for apoptosis induction. TGF- β -induced apoptosis include targets such as pro-apoptotic caspases and several members of the BCL2 family [8, 9]. In addition to its direct effect on epithelial tumor cells, TGF- β further controls tumor development by modulating the tumor microenvironment [48] and by regulating growth factors production by the surrounding stroma [9]. Furthermore, TGF- β suppresses immune and inflammatory processes. It inhibits CD8+ cytotoxic T cells, CD4+ T cells, macrophages, dendritic cells and NK cells and stimulates the generation of regulatory T cells and Th17 cells [48]. Disruption in TGF- β signaling may lead to inflammatory bowel diseases (IBD) which is a premalignant condition. Other tumor suppression functions of TGF- β include regulation of autophagy and senescence [48].

Evading the tumor suppressive mechanisms of the TGF- β pathway

The ability of tumors to escape the TGF- β -associated cytostatic program is a prerequisite for the consequent use of this same pathway for tumor promotion. There are multiple mechanisms by which tumors can escape the growth suppression effects mediated by TGF- β . One of these, exhibited mainly in gastrointestinal and head and neck cancers, is through inactivating mutations of the core elements of the TGF- β signaling pathway, the receptors, and the Smad genes [9, 49]. Loss of Smad4 does not only result in the loss of TGF- β tumor suppressive function, but may in fact switch TGF- β from a tumor suppressor to a tumor promoter [50]. Other types of cancer preserve these components but still inhibit only the TGF- β tumor suppressive arm by altering Smad-regulated genes that mediate the cytostatic program, which brings about failure of p15 induction and c-Myc suppression in response to TGF- β [8]. However, as tumors progress, epigenetic and genetic changes in the tumor cell and environment may switch the TGF- β pathway itself from a tumor suppressor to a tumor promoter [6, 47]. Mutations in the tumor suppressor p53 can change the TGF- β response. Oncogenic activation of Ras results in phosphorylation of mutant p53 which enables ternary complex formation between ligand-activated Smads2/3 and p63. This complex acts to suppress the transcriptional activation function of p63 and enables TGF- β to promote EMT, invasion and metastasis [51]. PDGF- β hypomethylation together with epigenetic downregulation of disabled (DAB2) enables TGF- β to promote proliferation [52, 53]. Another example in which TGF- β switches from tumor suppressor to tumor promoter is by JNK phosphorylation of the linker region of R-Smad, which is dependent on MAPK and promotes invasion. This is in contrast to the TGF- β dependent phosphorylation of R-Smad in its C-terminal region, which results in growth inhibition [54, 55]. Other possible mechanisms can be found in greater detail elsewhere [56].

TGF- β as a Tumor Promoter (Figure 1)

During the well-coordinated process of epithelial-mesenchymal transition (EMT), cells lose many of their epithelial characteristics, such as polarity and cell-cell contact, and they acquire mesenchymal properties, including enhanced motility and invasiveness. Interestingly, during EMT the epithelial cells acquire some stem cell characteristics. TGF- β is a regulator of the EMT process [57]. Overexpression of Smad3 or Smad4 results in increased EMT [58], while down-regulation of them or overexpression of Smad7 results in the opposite. The Smad3 and Smad4 adaptor protein β 2SP modulates Smad4 activation in the early stages of colon cancer to maintain epithelial structure and suppress EMT [7]. As mentioned, TGF- β has anti-inflammatory properties leading to tumor suppression; however, when the immunosuppressive effects of TGF- β become more dominant, the net effect is tumor progression [9]. TGF- β suppresses transcription of pro-apoptotic and cytolytic factors in cytotoxic T lymphocytes (CTLs) like granzyme A and B, perforin, interferon- γ and FAS ligand [49, 59]. TGF- β inhibits some of the functions of CTLs, CD8⁺ T cells, and natural killer cells; it also inhibits the secretion of IgA and the proliferation of B cells [60]. TGF- β drives the immune response from type 1 differentiated anti-tumor cells (neutrophils and macrophages) into the more immature type 2 cells which release more TGF- β -band IL-11 into the tumor environment, which result in a tumorigenic effect [61]. TGF- β enhances tumor invasiveness as well as angiogenesis. It promotes the production and secretion of matrix metalloproteases-2 (MMP-2) and MMP-9, and it down-regulates the expression of

the protease inhibitor TIMP [8, 9, 62, 63]. TGF- β can stimulate angiogenesis through its effects on angiogenic factors such as vascular endothelial growth factor (VEGF) and connective tissue growth factor (CTGF) [8, 9, 64]. TGF- β affects the metastatic process as well. Plasma levels of TGF- β are correlated to the presence of metastases in different types of cancer like CRC, pancreas and non-gastrointestinal tumors like prostate and breast cancers [47].

TGF- β in Colorectal Cancer

In CRC, the expression of TGF- β 1 is markedly increased [48, 65, 66]. While well differentiated to moderate differentiated, localized CRCs respond to TGF- β with growth inhibition, metastatic carcinoma cells proliferate after treatment with TGF- β [67-69]. Increased TGF- β levels within the primary tumor as well as high plasma levels of TGF- β correlate with poor prognosis in CRC patients [67, 68, 70]. In CRC, mutations of the TGF- β receptors and the Smad genes are common. T β RII mutations occur late in the adenoma to carcinoma sequence [71]. While about 30% of all CRCs exhibit T β RII mutations, these inactivating frameshift mutations can be found in 87% of tumors presenting microsatellite instability (MSI-H) [72]. T β RII mutations are more prevalent in the right colon than in rectosigmoid cancers, as MSI-H tumors [73]. T β RII and ACVR2A, another TGF- β component, were found to be the most frequently mutated genes in MSI-H CRC from patients with Lynch syndrome [74]. A recently published paper has shown that TGF- β signaling may still remain active in some MSI-H CRC cells despite the presence of frameshift mutations in the T β RII gene [75]. The exact mechanism of the contribution of T β RII mutations to CRC is still not known. Studies have suggested that inactivation of T β RII goes along with KRAS mutations to induce intestinal neoplasms in mice in a β -catenin independent pathway [68, 76]. Inactivation of T β RII also can increase expression of VEGFA, thereby increasing metastatic potential of CRC cells [77]. T β RI mutations are less frequent in CRC. Deletion of three alanine amino acids from a nine alanine stretch in the N-terminal side of T β RI (T β RI (6A)) is associated with increased cancer risk [78],[79] although this may not be a consistent phenomenon [68, 80].

Smad4 mutations occur in 8-30% of metastatic CRCs [9, 62, 81, 82] generally in the later stages of carcinogenesis. These mutations are associated with poor prognosis. Smad4 germline mutations as well as germline mutations in BMPR1A, another component of the TGF- β pathway, are associated with Juvenile polyposis syndrome, an autosomal dominant hamartomatous polyposis syndrome accompanied by CRC and gastric cancer [83]. Loss of Smad4 alters BMP signaling to promote CRC cell metastasis via activation of Rho and Rock [84]. Smad2 mutations are found in 3.6–5% of CRCs [48, 81, 85, 86] arising in the early stages of tumorigenesis [87] Smad3 mutations were identified in human CRC [81] and correlate with some animal models with predisposition to CRC [68]. Other genes in the TGF- β signaling pathway that are involved in CRC formation include Smad7, BMP2, BMP4 and GREM1.

TGF- β in Gastric Cancer

TGF- β 1 expression is increased in gastric cancer mucosa, pre-cancer gastric cells [88], and gastric mucosa of the patients' first-degree relatives [48, 89]. In patients with gastric cancer, high serum and cancer tissue levels [90] of TGF- β 1 are associated with lymph node involvement and poor prognosis. T β RII mutations appear mainly in progressive MSI-H cancers [48, 85, 91]. Although mutations in T β RI are less common in gastric cancer, transcriptional repression of this receptor by DNA methylation correlate with poor prognosis [92]. Haploid loss of Smad4 initiates gastric polyposis and gastric cancer in mice [93], which resemble juvenile polyposis [30, 31, 48]. Smad2 and Smad3 mutations have not been described in gastric cancer but the expression of Smad3 in gastric cancer tissue is low or even undetectable in 40% of human gastric cancer tissue [66, 94].

TGF- β in Pancreatic Cancer

TGF- β expression is increased in pancreatic cancer and associated with poor prognosis [48, 65, 95]. The activation of TGF- β receptor signaling in pancreatic carcinoma cells increases Smad3 phosphorylation and nuclear translocation to inhibit cell growth. Meanwhile, it also activates Smad7 to induce nuclear translocation and retention of β -catenin, which attenuates the inhibition of cell growth by nuclear Smad3 and activates VEGFA to promote vascularization and metastases [96]. TGF- β also causes a fibrous reaction, one of the hallmarks of pancreatic adenocarcinoma. Mutations in the TGF- β pathway have a critical role in the pathogenesis of most pancreatic cancers [97, 98]. T β RII mutations are involved in 4-7% of pancreatic cancers [82, 97, 99], while mutations in T β RI or ActR1B (type I receptor for activin) are found in 2% of them [48, 82, 99, 100]. However, the most common mutation of the TGF- β pathway in pancreatic cancer is in Smad4. Smad4, formerly called DPC4 (deleted in pancreatic cancer 4), is located at the 18q21 locus which is deleted in 60-90% of pancreatic cancers [97]. Homozygous deletion of this gene appears in 30% of pancreatic cancers while it is inactivated in another 20% [97, 100]. Losing the normal signaling of Smad4 may promote K-ras-driven malignant transformation of pancreatic duct cells [101].

TGF- β in Liver Cancer

Hepatocarcinogenesis is a multistep process. Most cases of HCC occur following chronic liver inflammation in a cirrhotic liver. TGF- β -mediated crosstalk between malignant hepatocytes and the surrounding stroma plays a dominant role in HCC formation. TGF- β is highly expressed in HCC [102]. TGF- β 1 levels are associated with disease progression and poor outcome [103]. The expression of TGF- β begins earlier during the inflammatory and cirrhotic phases when TGF- β has a key role in fibrogenesis. Alterations of TGF- β signaling affect each phase of HCC formation differently. While during the early phases TGF- β inhibits proliferation of premalignant hepatocytes, later it promotes stromal formation, EMT, and tumor invasion [54]. TGF- β signaling promotes HCC progression by acting as an autocrine or paracrine growth factor and by inducing microenvironment changes [104]. Secretion of TGF- β from stromal cells activates fibroblasts, affects T regulatory cells and acts on tumor initiating cells [104, 105]. A temporal TGF- β gene expression signature successfully discriminated distinct subgroups of HCC: "early" and "late" TGF- β signatures.

The early response pattern reflects the physiologic response of TGF- β , while the late response pattern is associated with prolonged TGF- β activation [106]. Tumors expressing late TGF- β -responsive genes displayed invasive phenotype and shorter survival. These cells express high of TGF- β and Smad7, and show significantly reduced Smad3 signaling [107]. In HCC, T β RII expression is reduced and the receptor is mutated. These are associated with poor prognostic signs [108, 109]. About 25% of malignant hepatocytes show decreased T β RII staining, compared to surrounding non-malignant hepatocytes [54]. This reduction in T β RII minimizes the anti-proliferative effect of TGF- β . However, most HCCs still express T β RII, and therefore are TGF- β sensitive. Cell lines that correspond to the late TGF- β response lack T β RI, have low levels of T β RII and do not demonstrate growth inhibition while challenged with TGF- β . These lines produce EMT-characteristic proteins, such as vimentin, suggesting that TGF- β -related EMT is independent of the expression of TGF- β receptors [54, 55, 106, 110]. One possible mechanism to the switch from early gene response to TGF- β (tumor suppression) and the late gene response (tumor promotion) is by JNK phosphorylation of the linker region of R-Smad. Although not as common as in pancreatic and colon cancers, Smad4 and Smad2 mutations are observed in HCC. 40-70% of β 2SP heterozygous mice develop HCC at the age of 12-15 months [111, 112]. Ubiquitination of β 2SP and Smad3 by PRAJA1, a RING finger protein, may interfere with TGF- β signaling and also lead to development of liver cancer [9, 113].

Therapeutic Targeting of TGF- β Signaling Pathway

Although the TGF- β signaling pathway has potent anti-proliferative capabilities, it also has tumor promoting effects. These dual effects of TGF- β are a major barrier for the development of drugs targeting this pathway. Therefore, the timing of treatment and selection of patients should be carefully considered before giving drugs which enhance or decrease TGF- β effect. Because high serum and high tissue TGF- β levels are associated with worse prognosis for GI cancers, it is reasonable to target TGF- β for treatment of these tumors. We will briefly describe the strategies for TGF- β inhibition and will give gastrointestinal cancer-related examples. Comprehensive reviews about targeting TGF- β for therapeutic purposes can be found elsewhere [9, 66]. Therapeutic strategies against TGF- β can be divided into three levels [9]:

- 1) Ligand level: Using antisense molecules for prevention of TGF- β synthesis.
- 2) Ligand-receptor level: using anti-ligand and anti-receptor monoclonal antibodies or soluble receptors for prevention of ligand-receptor interaction.
- 3) Intracellular level: signal transduction blockade by receptor kinase inhibitors.

Antisense Molecules

Antisense molecules are single-stranded oligonucleotides that bind complementary sequences on specific mRNA, thereby preventing their translation and accelerating its degradation [9, 61, 114]. AP12009 (Trabedersen, Antisense, Pharma) is an antisense molecule against TGF- β 2, which was used successfully on glioma cells and on a mouse model of pancreatic cancer. It was successfully tested in an open labeled Phase I/II study in patients with stage III/IV pancreatic cancer, malignant melanoma and CRC. Survival

analysis of pancreatic cancer patients revealed a median overall survival [60] of 13.4 months (n = 9). One patient had a complete response of liver metastases and was still alive after 75 months. The drug was safe and well tolerated, apart from thrombocytopenia observed in some patients [115]. AP11014 is another antisense molecules in pre-clinical trials for treatment of CRC and other non- gastrointestinal cancers [66].

Ligand-receptor Level

Three fully humanized monoclonal antibodies against TGF- β were developed by Genzyme and tried in clinical trials: GC-1008 (Fresolimumab), CAT-152 (Lerdelimumab) and CAT-192 (Metelimumab). None of these has been tested in clinical trials for gastrointestinal malignancies [9]. Soluble T β RII reduces pancreatic cancer metastasis in pre-clinical models [116], and soluble T β RIII inhibits the growth of human colon cancer cells [117]. However, these have not yet been studied in clinical trials.

Signal Transduction Blockade

Receptor kinase inhibitors are orally administered drugs which have been extensively studied recently. Most of the TGF- β -associated receptor kinase inhibitors act by inhibition of the catalytic ATP-binding site of T β RI [9]. LY2157299 (galunisertib, Eli-Lilly & Co) is the only TGF- β receptor kinase inhibitor currently in clinical trials. It is a T β RI kinase inhibitor that showed preliminary promising results in the treatment of glioma patients [118]. At present it has been tested in a Phase Ib/II in stage II-IV pancreatic cancer combined with gemcitabine versus gemcitabine plus placebo (NCT01373164). An interim analysis from a Phase II clinical trial in 109 HCC patients who have had disease progression on Sorafenib or were not eligible to receive it was presented at the 2014 ASCO meeting, showing alfa feto protein (AFP) decline of >20% in 24% of patients. The median overall survival was 36 weeks. In AFP responders, median overall survival was 93.1 weeks versus 29.6 weeks in non AFP responders. Interestingly, only 4 patients discontinued treatment due to adverse events [119]. Ki26894 and SB-435]are other T β RI inhibitors demonstrating positive effect in *in vitro* experiments using gastric cell lines [120], and CRC [121], respectively, but these have not been tested in clinical trials.

Foregut cancers with inactivated TGF- β signaling produce high levels of cyclin D1 and CDK4 expression. Our group as well as others have shown that CDK4 activation with disruption of TGF- β signaling is common in colon and hepatocellular cancers [112, 122]. These studies have led to a series of clinical trials targeting these molecules in progress. Clinical trial of CDK4 Inhibitors with/without a BRAF inhibitor is currently advancing for colon cancer (NCT01522989 and NCT01037790). ON123300, developed recently and currently in Phase I, is a potent multikinase inhibitor of 2 kinases, CDK4 and ARK5 [123]. The inhibitory activities of ON123300 and PD0332991 [124], a CDK4/6 inhibitor developed by Pfizer Pharmaceutical Corporation, currently in Phase I-III trials (depending on organ type) are being compared by our group.

Conclusions

The role of TGF- β as a tumor suppressor and tumor promoter is context dependent. Alterations of the TGF- β pathway are common in liver and gastrointestinal cancers and lead to tumor formation and metastases; its role in stem cells and cancer is also under active investigation. For instance, the effector role of the TGF- β pathway in suppressing Beckwith-Wiedemann syndrome (BWS), a human stem cell overgrowth disorder causally associated with an 800-fold increased risk of developing several types of tumors, has recently been described and investigated [41]. Examining the role of the TGF- β pathway in modulating stem cell differentiation and the cancers that ensue from these cells is an exciting component of future studies. Identifying novel strategies that target specific components of the TGF- β signaling pathway ranging from the inflammatory, fibrotic to the tumor suppressor and later tumor promoting metastatic aspects will lead to improved outcomes for lethal liver and gastrointestinal cancers.

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Highlights

- Alteration of TGF- β signaling pathway is associated with liver and gastrointestinal cancers.
- The function of TGF- β is context dependent. While in the gastrointestinal tract TGF- β functions as a tumor suppressor, it can also function as a tumor promoter.
- The dual effects of TGF- β are a major barrier for the development of drugs targeting this pathway.
- Few drugs targeting the TGF- β pathway have proven effective in clinical trials. Identifying novel strategies that target specific components of this pathway will lead to improved outcomes for lethal liver and gastrointestinal cancers.

Figure 1. TGF- β signaling in tumor suppression and promotion

TGF- β achieves its tumor suppressive and promoting effects through several mechanisms. An important suppressor mechanism is through regulation of cell proliferation. TGF- β upregulates the expression of p21 and p15 to inhibit cyclin-dependent kinase (CDK) and downregulates the expression of c-Myc and ID to drive cell proliferation. TGF- β also exerts its tumor promoting effect through induction of EMT by Smad3 and Smad4 upregulation; Examples of evasion of the immune system are by suppression of granzyme A, B, perforin, FAS ligand and IgA; angiogenesis by promotion of MMP2, MMP9, VEGF and CTCT; and metastasis.