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TGF-β Signaling in Liver, Pancreas, and Gastrointestinal Diseases and Cancer

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Abstract

Genetic alterations affecting transforming growth Factor–β (TGF-β) signaling are exceptionally common in diseases and cancers of the gastrointestinal system. As a regulator of tissue renewal, TGF-β signaling and the downstream SMAD-dependent transcriptional events play complex roles in the transition from a noncancerous disease state to cancer in the gastrointestinal tract, liver, and pancreas. Furthermore, this pathway also regulates the stromal cells and the immune system, which may contribute to evasion of the tumors from immune-mediated elimination. Here, we review the involvement of the TGF-β pathway mediated by the transcriptional regulators SMADs in disease progression to cancer in the digestive system. The review integrates human genomic studies with animal models that provide clues toward understanding and managing the complexity of the pathway in disease and cancer.

Keywords

Transforming Growth Factor–β; Digestive System; Cancers

Transforming growth Factor–β (TGF-β) signaling is crucial to homeostasis of epithelial cells, stromal compartments, and immune cells in the liver, pancreas, and gastrointestinal

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(GI) system. Altered activity contributes to the development of tissue-specific diseases and progression to malignancy. The importance of TGF-β signaling in digestive system health is epitomized by the high frequency of genomic alterations in genes encoding proteins in the TGF-β pathways in cancers of the digestive system: 40% of GI cancers have such alterations,[1] 38% of liver cancers,[2] and 50% of pancreatic cancers.[3]

Multiple types of cells both produce and respond to TGF-β, creating a complex network that involves epithelial cells, tumor cells, immune cells, and stromal fibroblasts. This complex network both contributes to disease and changes over time, enabling TGF-β to have both tumor-suppressing and tumor-promoting or tumor-enabling roles. We focus on TGF-β signaling through SMAD proteins (Figure 1). Of the more than 30 members of the TGF-β superfamily, the TGF-β and bone morphogenetic protein (BMP) families are particularly relevant. The 12 serine/threonine kinase receptors form a heterotetrameric complex consisting of two type I receptors and two type II receptors; each receptor complex phosphorylates a specific set of SMADs. Phosphorylated SMADs form complexes, which translocate into the nucleus to regulate target gene expression. TGF-β1, TGF-β2, and TGF-β3, the TGF-β sub-family, signal through receptor complexes comprised of TGFBR1 and TGFBR2 to phosphorylate SMAD2 and SMAD3.

TGF-β proteins are secreted as inactive procomplexes consisting of the TGF-β dimer and latency-associated peptide. Release of active TGF-β can occur through either proteolytic mechanisms or allosteric mechanisms. Although the interaction of the procomplex with latent TGFb binding proteins results in deposition in the extracellular matrix (ECM);[4,5] association with glycoprotein-A repetitions predominant protein enables the procomplexes to bind to the cell surface of various immune cells.[6] Activation of the latent procomplexes can involve proteolysis of the ECM or allosteric interactions, such as those mediated by integrins.[7]

SMADs represent a regulatory family: 5 receptor-activated SMADs, SMAD1, SMAD2, SMAD3, SMAD5, and SMAD9 (also referred to as SMAD8); 1 co-mediator SMAD, SMAD4, which forms complexes with the receptor-activated SMAD dimers; and 2 inhibitory SMADs, which block SMAD activation or signaling. In addition to ligand-activated receptors, other proteins influence the activity of receptor-activated SMADs. SMAD-mediated transcriptional activity and target gene selectivity are regulated by various adaptors in both the cytoplasm and nucleus. Active SMAD complexes interact with other proteins to achieve target gene specificity and context-specific outcomes. TGF-β/SMAD signaling networks are complex, context-dependent, and critical for adult tissue homeostasis and immune function.

Transforming Growth Factor–β/SMAD Signaling in Diseases of the Digestive System and the Progression to Cancer

Throughout the digestive system, altered function of TGF-β is implicated in disease progression to cancer. The main text describes the roles of TGF-β1, TGF-β2, and TGF-β3; the Supplementary Material discusses BMP signals and celiac disease and includes

a comprehensive mouse models table (Supplementary Table 1), and table of reviews (Supplementary Table 2).

Barrett's Esophagus and Esophageal Cancer

Barrett's esophagus (BE) results from the replacement of squamous epithelium by precancerous tissue (dysplasia). BE is often associated with gastroesophageal reflux disease, is more common in men, has an increasing incidence, and is associated with increased risk of developing esophageal adenocarcinoma (EAC).[8] Impairment of TGF-β signaling is indicated by markedly reduced *SMAD4* expression in all stages of BE from metaplasia to both low- and high-grade dysplasia, and the expressions of *TGFBR2, TGFB1,* and *SMAD4* are lower in EAC compared to normal esophagus.[9,10] In >80% of BE and EAC samples, signaling by TGF-β is impaired, and genomic alterations in members of the superfamily of TGF-β pathway components occur in 65% of EAC.[1] Frequently altered in BE and EAC are *TP53* (encoding the tumor suppressor p53) and *CDKN2A* (encoding the cell cycle inhibitor p16). The combination of such driver mutations with loss of function of members of the TGF-β pathways may predispose BE to transition to cancer (Figure 2).[1]

For *SMAD4*, loss of heterozygosity of region containing *SMAD2* and *SMAD4* on chromosome 18q is common in approximately 70% of EACs associated with BE.[11,12] This loss of heterozygosity occurs in approximately 30%–70% of patients with premalignant BE, suggesting that this is an early event in neoplastic transformation.[12] Methylation of the *SMAD4* promoter is another mechanism of reducing *SMAD4* expression in both BE and EAC.[9] *SMAD4* genomic alterations are more common in EAC (24%) than in esophageal squamous cell carcinoma (8%).

A switch in SMAD3-regulated genes in response to loss of SPTBN1[13] suggests that loss of SMAD4 could also induce a switch in target gene regulation to induce a neoplastic transcriptional profile. Consistent with a target gene-switching mechanism, analysis of transcript abundance biopsy tissue from EAC patients reveals enhanced expression of genes in either 1 or both of 2 pathways in approximately 70%–80% of the samples—JNK–JUN pathway and the TGF-β1–SMAD2/SMAD3 pathway.[14] In cultured cells, TGF-β1–stimulated growth is SMAD4-independent and SMAD2/SMAD3-mediated signaling switches from growth-inhibiting to growth-promoting as the cells transition from dysplasia to neoplasia. Additional evidence supporting a switch from tumor-suppressing to tumor-promoting in TGF-β signaling comes from a study of the expression of genes in BE and EAC stromal tissue.[15] Increased expression of inflammatory genes, such as those encoding interleukin (IL)-6, C/EBPb, and COX-2, and genes induced by TGF-β signaling, such as *TSP1*, *POSTN*, and *TMEPAI*, in BE fibroblasts occurs in the patients that progressed from metaplasia to cancer and is associated with poor outcomes.[15] Induction of *POSTN*, which encodes a protein that promotes cell motility, stimulates invasive behavior of esophageal cancer cells in culture. However, knockout of *TGFBR2* in fibroblasts and dendritic cells (DCs) using the *FSP1* promoter triggers loss of parietal cells and development of squamous cell carcinoma in the forestomach (similar to human esophagus).[16] Thus, either increased or decreased TGF-β signaling in the stromal fibroblasts can contribute to neoplasia.

Gastritis, Infection, and Gastric Cancer

The risk of developing stomach cancer is associated with inflammation. Infection with *Helicobacter pylori* or chronic atrophic gastritis results in inflammation and tissue injury, which are associated with epithelial cell metaplasia. Inflammation in *Helicobacter*-infected mice is partially controlled by TGF-β1 produced by DCs.[17] Mice with DC-specific *TGFB1* knockout develop severe gastritis and exhibit increased metaplasia, despite reduced colonization by the bacteria (Table 1). In patients with *H pylori*, interferon-gamma inhibits TGF-β signaling by increasing SMAD7, and exposing biopsy tissue to a SMAD7 antisense molecule restores signaling.[18] Thus, TGF-β1 signaling is important for limiting metaplastic changes associated with *H pylori*–induced chronic gastritis (Figure 2). This is consistent with a tumor-suppressing role before neoplastic transformation.

Once the gastric epithelia become neoplastic, TGF-β signaling becomes hyperactivated, suggesting a switch from tumor-suppressing to tumor-promoting or tumor-supporting. Activation of a TGF-β signaling transcriptional profile is higher in gastric cancer (GC) compared with intestinal metaplasia of the stomach.[15] Furthermore, biopsy samples reveal high abundance of TGF-β1 and TGF-β3 in GC compared with normal tissue.[19] However, a mouse model with *FSP1*-mediated knockout of *TGFBR2* shows that loss of stromal TGF-β signaling can also lead to tumorigenesis of specific epithelial populations, including those of the stomach. Consistent with this finding in mice, patients with SMAD7-positive tumors had a worse survival rate than those with SMAD7-negative tumors, suggesting that impaired TGF-β signaling is also associated with poor outcomes in some patients, and underscoring the complexity in considering whether TGF-β signaling is tumor suppressive or tumor enabling.[20]

Alterations in TGF-β signaling are associated with metastasis of GC. Increased or decreased signaling occurs in multiple cells of the tumor microenvironment. A patient with GC that metastasized to the ovaries had a biallelic loss-of-function genetic alteration in *TGFBR2*.[21] Subsequent studies with mouse organoids in vitro and using the cells in a mouse xenograft model show that loss of *TGFBR2* in the context of genetic knockout of *CDH1* (encoding E-cadherin) and *TP53* results in metastatic phenotypes. GCs are molecularly diverse, with each subset having different connections to TGF-β signaling[22]: 9% are positive for Epstein-Barr virus (EBV), 22% are associated with microsatellite instability (MSI), 20% are genomically stable, and 50% exhibit chromosomal instability. EBV-positive GCs have high DNA hypermethylation, especially of the *CDKN2A* promoter, and mutations in *PIK3CA*, encoding the catalytic subunit of PI3K.[22] Although TGF-β1 stimulates apoptosis of GC cell lines, introduction of the EBV oncoprotein LMP2A blocks this apoptotic effect by stimulating PI3K-mediated activation of AKT.[23] EBV-infected gastric epithelial cell lines produce TGF-β1 and respond to this signal by activating EBV gene expression.[24] Thus, ineffective TGF-β1–mediated apoptosis and ongoing inflammation due to viral reactivation may contribute to progression of EBV-positive GC. In MSI GC, mutations are common in genes that are associated with increased susceptibility to altered TGF-β signaling, or are core to the TGF-β pathway—*TP53* (50%), *PIK3CA* (12%), *CDH1* (11%), *SMAD4* (8%), and *SMAD2* (2%).[22] Mutations in *TGFBR2* occur in GCs associated with either MSI or replication errors.[25] Reduced expression of *TGFBR2* is associated with a high frequency

of MSI at this gene (approximately 52%) in GC.[26] Like the other subtypes of GC, genes associated with increased susceptibility to altered TGF-β signaling were common in the genomically stable and the chromosomal instability types. In the genomically stable tumors, 37% had mutations in *CDH1*; in the chromosomal instability tumors, 71% had mutations in *TP53*. Thus, even though these subtypes were not associated with mutations in genes of the TGF-β pathway, these cancers can arise through impairment of TGF-β–mediated tumor-suppressor signaling.[22]

Inflammatory Bowel Disease, Hereditary Colon Cancer Syndromes, and Colorectal Cancer

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis (UC), is characterized by chronic or recurring inflammation of the GI tract. IBD is associated with disrupted immune homeostasis that involves T cells and innate lymphoid cells. Through genome-wide association studies, more than 200 risk loci are associated with IBD or the subtypes of Crohn's disease and UC.[27,28] Of particular relevance here is the identification of loci containing *SMAD2*, *SMAD3*, *SMAD4*, and *SMAD7* (Figure 2). A *SMAD3* locus was found to be associated with IBD.[28] Single-nucleotide polymorphisms in *SMAD2*, *SMAD3*, *SMAD4*, and *SMAD7* were identified specifically in patients with UC, not those with Crohn's disease.[29]

Biallelic loss-of-function mutations in *TGFB1* lead to severe infantile IBD and central nervous system defects.[30] Consistent with a loss of immune homeostasis, the lamina propria of 1 patient had decreased frequency of multiple subpopulations of T cells, including regulatory T cells (Tregs), T helper (Th) type 1 cells, and Th17 cells. These findings in children support the translational validity of the observations that mice with global *SMAD3* knockout[31] spontaneously develop IBD and mice with a global *TGFB1* knockout develop severe multiorgan inflammatory disease that includes the intestine.[32] Mice in which TGF-β is induced by oral administration of haptens to induce "tolerance" or before induction of experimental colitis with the same molecules, results in a reduction in inflammation and colitis-associated symptoms.[33]

Intestinal epithelial cells release proinflammatory signals in response to IL-22, which was transcriptionally up-regulated in biopsies from patients with active Crohn's disease.[34] In the presence of IL-6 and TGF-β, Th17 cells produce IL-17 and transcriptionally suppress *IL22*.[35] Thus, context-dependent TGF-β signaling can provide an anti-inflammatory input and loss of this signal can contribute to intestinal inflammation. Mice with either a T cell–specific deletion of *TGFB1* [36] or introduction of dominant-negative *TGBR2* specifically in T cells[37] develop IBD. DC-specific *TGFBR2* knockout also results in multiorgan inflammatory disease, which, in the colon, involves increased production of pro-inflammatory cytokines, reduced function of Tregs,[38] an altered microbiota, goblet cell loss, and a reduction in the mucous layer.[39] Together these mouse studies indicate that TGF-β1 signaling in DCs is important not only for maintaining T cell homeostasis, but also for enabling the differentiation of mucous-producing goblet cells that are critical for maintaining a healthy epithelium that is continuously exposed to enteric microbes.

Analysis of tissue and experiments with explanted tissue reveals increased abundance of SMAD7, anti-SMAD, and strongly reduced phosphorylated SMAD3 in the Crohn's disease

tissue and a moderate reduction in UC tissue.[40] Exposing immune cells isolated from the lamina propria of these tissues to SMAD7-specific antisense oligonucleotides increases TGF-β1–stimulated SMAD3 phosphorylation. Furthermore, these antisense oligonucleotides reduce interferon-gamma and tumor necrosis factor–α in the explanted tissue from patients with Crohn's disease, consistent with a shift from pro-inflammatory T cells (Th1 and Th17 cells) to Tregs in response to increasing TGF-β1 signaling.[41] Studies with mice support the increase in SMAD7 in IBD and the effectiveness of SMAD7-targeted antisense oligonucleotides in enabling TGF-β1–mediated SMAD3 activation and reducing inflammation.[42] These studies resulted in clinical trials of an orally available SMAD7 antisense oligonucleotide (mongersen) for active Crohn's disease, which yielded positive results in phase II[43,44] but failed in phase III.[45]

Small intestinal fibrosis and stricture formation often occurs in Crohn's disease. TGF-β signaling regulates many genes involved in fibrosis, such as those encoding collagens and matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). TGF-β also stimulates fibroblasts to become myofibroblasts, which both produce proteins that alter the ECM and have contractile properties. Tissue injury and the activity of MMPs, such as occurs in chronic inflammatory conditions like IBD, release latent TGF-β from the ECM, and integrins mediate local activation of latent TGF-β. Tissues from both patients with UC[46] and patients with Crohn's disease[47] exhibit changes in TGF-β signaling and pro-fibrotic pathways, including MMPS, TIMPs, and integrins.[48]

Myofibroblasts contribute to fibrosis and stenosis. The phenotypes of the myofibroblasts associated with Crohn's disease or UC are different. Myofibroblasts from fibrotic Crohn's disease tissue exhibit increased expression of *TIMP1* and release higher amounts of TIMP1 into the medium compared with myofibroblasts cultured from normal tissue or from patients with UC.[49] Intestinal myofibroblasts from patients with Crohn's disease or UC exhibit differential production of TGF-β isoforms; those from Crohn's disease tissue produce all 3 isoforms (TGF-β1, TGF-β2, TGF-β3), those from UC tissue produce primarily TGF-β1 and TGF-β3, and those from normal tissue produce mostly TGF-β3.[50] Despite producing all 3 isoforms, only the myofibroblasts from the Crohn's disease tissue are independent of the autocrine effects of TGF-β for proliferation in culture. In culture, TGF-β3 inhibits proliferation the least and TGF-β2 is the least effective in promoting cell migration.[50]

Differences in TGF-β signaling exist in the mucosal tissue and myofibroblasts from strictured and nonstrictured regions of patients with fibrostenosing Crohn's disease, from inflamed tissue from patients with nonfibrostenosing disease, and from normal intestine.[51] Strictured areas have the highest activity of TGF-β signaling and the highest amounts of TIMP1 and collagen, whereas inflamed areas from patients with nonfibrostenosing disease have the highest amount of SMAD7, MMP12, and MMP3. These differences relate to the myofibroblasts in the tissues and suggest that myofibroblast SMAD7 abundance may regulate TGF-β signaling at different stages of the disease process or contribute to the type of disease present.

Intestinal microbial populations are altered in patients with IBD.[52–54] Of particular relevance to TGF-β signaling are the reductions in the butyrate-producing *Clostridium*

populations and *Bacteroides* populations and in the increase in *Enterobacteriaceae*. Studies with mice suggest that the reduction in butyrate-producing species impairs intestinal epithelial cell TGF-β1 production,[55] as well as reduces the responsiveness of T cells to Treg-inducing TGF-β1 signals by affecting histone acetylation.[56] The consequent change in *FOX3P* expression shifts the T-cell balance away from the inflammation-suppressing Treg, contributing to colitis. Combined with the high abundance of SMAD7 in the immune cells from inflamed IBD patient tissue,[40] this dysbiosis likely contributes to the hyperinflammatory conditions by further impairing immuno-suppressive TGF-β signaling. Mouse studies suggest that impaired TGF-β signaling in DCs could contribute to the increase in *Enterobacteriaceae*.[39] Other studies in mice show that dysfunction of the TGF-β pathway in immune cells[57] is associated with IBD susceptibility in response to commensal microbes. An attractive concept for treating IBD is a therapy that restores a microbial community to enable TGF-β responsiveness, promote Treg differentiation, and limit inflammation.[58]

Inflammation and microbial dysbiosis are risk factors for colorectal cancer (CRC), which includes both colon adenocarcinoma and rectal adenocarcinoma. Furthermore, mutations in any of 43 genes encoding components in the superfamily of TGF-β pathways are present in 65% of colon adenocarcinomas and 50% of rectal adenocarcinomas.[1] Various studies in mice provide mechanistic connections between changes in the intestinal microbiome or susceptibility to microbial infection, gut inflammation, and cancer development (Figure 2). Mice with global *TGFB1* knockout that are also genetically immunodeficient (*Tgfb1*$^{-/-}$*Rag2*$^{-/-}$) require gut microbes for inflammation-induced adenocarcinoma and progression from colitis-associated inflammation to cancer.[59] *SMAD3* global knockout mice housed under normal conditions, but not those lacking *Helicobacter* in the GI tract, develop metastatic CRC.[60] The introduction of *Helicobacter* induced inflammation that progressed to mucinous adenocarcinoma.[61] Mice heterozygous for *SMAD4* and for the gene encoding the SMAD3 adaptor protein SPTBN1 spontaneously develop CRC, and these mice exhibit an altered gut microbiota that is enriched in microbial populations associated with human CRC.[62] The CRC-associated mutants exhibit enhanced ability to inhibit TGF-β signaling in cultured cells.[62,63]

The high frequency of alterations in genes in the components of the superfamily of TGF-β pathways in CRC suggests that these pathways prevent these cancers or that altered activity enables these cancers to progress in the presence of gut microbiota. *SMAD3*$^{-/-}$, *SMAD4*$^{+/-}$, *SPTBN1*$^{+/-}$ double heterozygous mice develop CRC, and T cell–specific, not epithelial cell–specific, *SMAD4* knockout causes CRC.[60,62,64,65] The T cell–specific *SMAD4* knockout models phenocopy familial juvenile polyposis syndrome, some cases of which are associated with germline mutations in *BMPR1A* or *SMAD4*[66] and increased risk of CRC. Furthermore, disruption of *TGFBR2*, specifically in intestinal epithelial cells; *SMAD4* heterozygosity; or *SMAD3* knockout accelerate the development of invasive, inflammation-associated colorectal adenocarcinomas in mice with *APC* mutations (Supplementary Table 1).[67–70] Thus, in the context of *APC* mutations, suppression of TGF-β signaling either in the intestinal cells or the T cell population contributes to CRC development.

APC mutations cause aberrant Wnt signaling by constitutively activating b-catenin (encoded by *CTNNB*). Various classifications exist for CRC, one of which defines 4 main subtypes: 13% exhibit epithelial differentiation with metabolic dysregulation (CMS3); 14% are associated with MSI and immune cells with Th1 and cytotoxic phenotypes (CMS1); 23% are associated with a mesenchymal phenotype that includes high activity of TGF-β signaling, stromal invasion, increased abundance of ECM proteins, and angiogenesis (CMS4); and 37% exhibit epithelial differentiation with increased Wnt and MYC signaling, frequent amplifications in oncogenes, and losses in tumor suppressor genes (CMS2). CMS4 is also associated with late-stage, metastatic tumors, suggesting that by stimulating epithelial-to-mesenchymal transition (EMT), TGF-β signaling functions as a tumor promoter in this subtype.

Severe, invasive CRC develops in mice with intestinal stem cell–targeted deletion of genes encoding APC, TGFBR2, and TRP53, and introduction of an activated KRAS mutant.[71] Although the tumors could not respond to TGF-β signals, TGF-β signaling in the stroma was high at the invasive margins of the tumors, which correlated with the immune "cold" character of these tumors, suggesting that high TGF-β signaling may interfere with T cell infiltration of the tumor. Transcriptional profiling of tumors transplanted into the ceca of syngeneic mice, but not those grown in culture, revealed that these tumors resemble the CMS4 subtype. Single-cell analysis of patient-derived CRC reveals enrichment in stromal cells, especially cancer-associated fibroblasts (CAFs), in the subset of patients with poor prognosis.[72,73] High expression of *TGFB1* and *TGFB3*, along with induction of a TGF-β response transcriptional profile in fibroblasts, is also associated with poor prognosis. Multiple mouse models reveal how the stroma contributes to the protumor effects of TGF-β signaling in CRC: Mice with tumors derived from a patient with inactivating mutations in both *TGFBR2* alleles,[73] mouse tumor organoids with intestinal cell-targeted mutations,[71] or patient-derived tumor organoids with high expression of *TGFB1*.[72] In these models, blocking stromal TGF-β signaling with the selective TGFBR1 inhibitor Galunisertib (LY2157299) reduces metastatic burden.[71–73] Thus, independently from effects on the cancer cells, TGF-β signaling in the tumor microenvironment plays a key role in both tumor development and progression to highly invasive CRC.

SMAD7 has dual roles in development and progression of CRC, reflecting the cell-specific effects of TGF-β signaling in cancer. Genome-wide association studies reveal that *SMAD7* variants are associated with increased risk of developing CRC.[74] Overexpression of SMAD7 in non-tumorigenic CRC-derived cell line responsive to TGF-β signaling results in tumorigenicity and liver metastasis in nude mice, along with antagonism of TGF-β–mediated growth inhibition and apoptosis.[75,76] These studies indicate that SMAD7 interferes with a tumor-suppressive role for TGF-β signaling within the tumor cells. In contrast, mice with T cell–specific overexpression of SMAD7 develop fewer colitis-associated tumors, despite exhibiting increased susceptibility to chemically induced colitis.[77] These mice are also resistant to tumor development by subcutaneously transplanted syngeneic CRC cells.[78] The protective role of SMAD7 against CRC in these models involves interferon-gamma, tumor necrosis factor–α, and infiltration of T cells into the tumor microenvironment. These studies indicate that TGF-β signaling within T cells provides a tumor-enabling, immune-suppressive microenvironment.

Nonalcoholic Fatty Liver Disease, Nonalcoholic Steatohepatitis, and Hepatocellular Carcinoma

Nonalcoholic fatty liver disease (NAFLD) ranges from benign steatosis to aggressive nonalcoholic steatohepatitis (NASH). NAFLD often progresses to fibrosis, cirrhosis, liver failure, or hepatocellular carcinoma (HCC). The amount of fibrosis is the key clinical indicator of outcomes of NAFLD, independent of histologic evidence of NASH.[79,80] In NALFD, there is an infiltration of immune cells and activation of Kuppfer cells, all of which produce TGF-β[81–83] and also respond to TGF-β. Although TGF-β signaling is critical for liver repair, in NAFLD the initiating condition does not resolve appropriately. Thus, the carefully orchestrated TGF-β response fails to move from the initial phase to the resolution phase.

In the presence of injury or inflammation, like that in NAFLD or NASH, TGF-β1 induces apoptosis and inhibits proliferation of hepatocytes[84,85] and activates hepatic stellate cells.[86–88] Studies with cultured cells and mice indicate that TGF-β1 also promotes the differentiation of mesothelial cells into hepatic stellate cells and myofibroblasts during chemically induced liver fibrosis.[89] The activation of hepatic stem cells and formation of myofibroblasts are pro-fibrotic consequences of TGF-β1 signaling: Both activated hepatic stellate cells and myofibroblasts produce pro-inflammatory signals, remodel the ECM, and deposit collagen.[86]

This remodeling of the ECM can release TGF-β latent complexes. This activated TGF-β from the ECM can synergize with inflammatory mediators, such as IL-22, to activate hepatic stellate cells.[90] Both IL-22 and IL-17 are associated with fibrotic liver disease and enhance responsiveness to TGF-β signaling in hepatic stellate cells,[90,91] connecting inflammation and TGF-β signaling to progression of fibrotic liver disease. The chronic inflammation and fibrotic tissue damage associated with NAFLD and NASH are risk factors for HCC. As treatments become more available for viral-associated HCC,[92,93] the frequency of NAFLD- and NASH-associated HCC will increase. Fibrosis results in a stiffer ECM, which enhances TGF-β1 autocrine signaling and triggers EMT in HCC cells in culture and enhances HCC metastasis in a rat model.[94] Furthermore, a stiffer matrix also induces HCC cells in culture to express markers of cancer stem cells.[95]

The role of TGF-β in the progression from inflammation to fibrosis to HCC is complex; TGF-β1 signaling has different effects on multiple cells at various stages of progression to neoplasia (Figure 3).[93] Studies with mice show that TGF-β1 functions as tumor suppressor, limiting hepatocyte proliferation under normal conditions, but enabling chemically induced HCC in the heterozygous state[96] or upon expression of a dominant-negative TGFBR2.[97] Impaired TGF-β signaling is linked to the development of cancer stem cells in HCC, consistent with an early tumor-suppressing role for TGF-β. Studies with cultured hepatocyte progenitor cells reveal suppression of SMAD3-mediated gene expression downstream of TLR4, a receptor involved in innate immune responses, contributes to the induction of cancer stem cell properties.[98] Altered SMAD3 function that occurs in mice heterozygous for the SMAD3 adaptor protein SPTBN1 results in spontaneous development of fibrosis and HCC, which is associated with hyperproliferative endothelial cells that result in aberrant angiogenesis.[99] Crossing these *SPTBN*$^{+/-}$ with mice lacking *ITIH4*, which is stimulated by

IL-6 and encodes a serine protease, suppressed cancer stem cell generation and HCC.[100] Tissue from living donor liver–transplanted specimens contains small groups of cells that are positive for stem cell markers and for SPTBN1 and TGFBRII,[100] indicating that healthy tissue contains a subset of cells poised to become cancer stem cells should they lose TGF-β–mediated inhibition.

Mouse studies also support a tumor-promoting role for TGF-β1 in HCC. Overexpression of TGF-β1 alone or in combination with MYC, both specifically in hepatocytes, results in HCC and accelerates chemically induced HCC in mice.[101] Combined hepatocyte-targeted overexpression of TGF-β1 and cyclin D1 in mice results in initial inhibition of hepatocyte proliferation, followed by development of highly invasive HCC, suggesting that TGF-β1 signaling switches from tumor-suppressing to tumor-promoting (or enabling) in this noninflammatory HCC model.[102] Analysis of HCC cell lines reveals diverse outcomes in response to TGF-β1. Some cells respond with EMT or partial EMT, and cells that respond with partial EMT have increased expression of genes indicative of stem cells.[103] Furthermore, knocking down TGFBRI reduces these invasive malignant properties of the cells. Another study shows that some HCC cell lines respond with apoptosis, some with proliferation, and some with inhibition of proliferation.[104] Furthermore, by altering the immune environment to one that is immunosuppressive, TGF-β1 also promotes progression of HCC and immune evasion that may be exacerbated by metabolic changes associated with NAFLD.[105,106]

Genetic and transcriptomic data reveal profiles consistent with an activated or an inactivated TGF-β signaling system in patient HCC tissue.[2] Patients with an inactivated TGF-β signature have the worst prognosis. Consistent with the TGF-β signal predominantly affecting stromal cells, expression of genes associated with activated fibroblasts was increased in HCC with the activated TGF-β signature. Like CRC, the TGF-β inactivated signature was associated with HCC with mutations in genes associated with DNA repair. HCC patients with TGF-β–positive gene signature (one dependent on TGFBR2) can be divided into 2 groups based on the temporal profile of the response to TGF-β: one with an “early” TGF-β signature and the other with a “late” TGF-β signature.[107] HCC patients with a late TGF-β signature display worse prognosis and overall survival, consistent with TGF-β signaling having different outcomes at different stages of cancer progression.

Despite tumor-suppressing roles for TGF-β, interest exists for inhibiting TGF-β signaling in chronic liver disease to prevent fibrosis and progression to invasive HCC. This concept is supported by mouse studies. Mice with hepatocyte-specific overexpression of SMAD7 exhibit protection from chemically induced fibrosis and liver damage.[108] Mice with hepatocyte-specific deletion of SMAD7 have increased susceptibility to chemically induced HCC.[109,110] However, context is important: Mice with SMAD7, MYC, and MCL overexpression or SMAD7 overexpression and activated AKT have accelerated development of liver cancer.[111]

HCC patients treated with sorafenib, an inhibitor of multiple tyrosine kinases, and who have high circulating TGF-β1 have worse prognosis and shorter survival than patients with low circulating TGF-β1.[2,112] Various preclinical studies show beneficial effects

of LY2109761, a TGFBR1/TGFBR2 inhibitor,[113] in blocking tumor growth and tumor angiogenesis in xenograft models of HCC[114] and blocking HCC cell invasiveness.[115,116] HCC tissues exposed to galunisertib, alone or in combination with sorafenib, exhibit reduced proliferation markers and increased apoptotic markers.[117] Galunisertib reduces stem cell markers in HCC cell lines and patient tissue samples.[118]

Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDAC), the most common and deadly form of pancreatic cancer, typically follows a predictable morphologic and genetic course that converts normal epithelial cells to noninvasive pancreatic intraepithelial neoplasia (PanIN) that progresses to PDAC. The median survival of patients with PDAC is 6 months, and the 5-year survival rate is approximately 6%.[119] The relative risk of developing PDAC is high in individuals with germline mutations in *STK11* (also known as *LKB1*, encoding a serine/threonine kinase), *PRSS1* (encoding a serine protease associated with hereditary pancreatitis), and *CDKN2A*. Inactivating *TP53* and *SMAD4* mutations occur in approximately 50% of these cancers and activating *KRAS* mutations in >90%.[3,120] Loss of SMAD4 occurs in later stages in the progression of PanIN to PDAC.[121]

The TGF-β pathway likely plays a role in the progression to malignant cancer through loss of the tumor-suppressing activity, such as occurs with combined activation of KRAS and inactivation of SMAD4 (Figure 3). Mice with pancreas-specific expression of $KRAS^{G12D}$ and pancreas-specific heterozygous or homozygous knockout of *TGFBR2* rapidly develop invasive PDAC.[122] Furthermore, the tumors also contained *CDNK2A* genomic alterations,[122] consistent with progressive acquisition of enabling mutations resulting in the progressive phenotypic switch from noninvasive PanIN to invasive PDAC.[123] Cell culture and mouse xenograft studies indicate that loss of SMAD4 activity or acquired resistance to TGF-β does not alter proliferation of human pancreatic ductal epithelial cell line expressing an activated KRAS mutant. However, loss of TGF-β responsiveness enhances invasive and metastatic phenotypes, consistent with loss of TGF-β responsiveness contributing to the later stages of progression to malignant, aggressive cancer.[124] Thus, aberrant or loss of TGF-β signaling within the cancer cells appears to serve a cancer-enabling or cancer-progressing function rather than a tumor-initiating function in the context of PDAC. Comparison of PanIN triggered by chemically induced pancreatitis in mice with pancreas-specific expression of an activating KRAS mutant in the presence or absence of SMAD4 knockout shows that TGF-β signaling induces apoptosis in signaling-competent cells through induction of a lethal EMT program.[125] The failure of PanIN to progress to PDAC in the context of functional TGF-β–SMAD signaling provides an explanation for the high-frequency *SMAD4* mutations in PDAC patients.[125] Consistent with a tumor-suppressing role of TGF-β signaling early in PDAC development, pancreas-specific expression of SMAD7 in mice disrupts TGF-β signaling and promotes PanIN.[126]

However, many PDAC tumors exhibit high amounts of TGF-β1, TGF-β2, or TGF-β3, or a combination thereof, with high amounts of TGF-β2 particularly associated with advanced tumor stage and high amounts of each correlated with worse outcome.[127] Reduced expression of the inhibitory *SMAD7* gene in patients is associated with poor prognosis.[128]

Patients with higher serum TGF-β before treatment tend to have better treatment outcomes, and a concentration that is higher after treatment than at diagnosis correlates with patients that progress.[129] Thus, TGF-β signaling may enable disease progression through effects on the tumor microenvironment.

Later stages of PanIN or PDAC are associated with a transcriptional profile of inactivated T cells and populations of immune-suppressing innate immune cells, consistent with the established immune-suppressing effects of TGF-β signaling.[130,131] PDAC has a desmoplastic stroma, consistent with TGF-β–induced myofibroblasts or activated pancreatic stellate cells.[130,132] At least 2 populations of CAFs are identified by transcriptional profiling,[131,132] an inflammatory type and a myofibroblast type. Studies with mouse PDAC organoids and pancreatic stellate cells shows that TGF-β signaling drives the myofibroblast phenotype and suppresses the inflammatory phenotype.[133] Tissue samples reveal differences between the immune cell populations in PDAC with different stromal properties, consistent with communication between the CAFs and the immune cells.[134]

Studies of pancreatic cancer cells and xenografts in mice found a reduction in tumor growth in response to kinase inhibitors of the TGFBR1/TGFBR2 complex, and this reduction is associated with less fibrosis and increased immune cell infiltration.[135,136] Mouse PDAC models show that combination treatment with galunisertib to inhibit the TGF-β receptor and lapatinib, an inhibitor of epidermal growth factor receptors (EGFR and HER2), reduces tumor growth and metastasis by inhibiting lymphangiogenesis and angiogenesis.[137] These results are consistent with finding a pro-angiogenic transcriptional profile in patient samples, angiogenesis in patient-derived xenografts, and angiogenesis in a PDAC mouse model.[138] Studies with cultured cells and mouse xenografts show that LY2109761, an inhibitor of the kinase activity of both TGFBR1 and TGFBR2, has synergistic anti-tumor effects with gemcitabine and reduces metastasis.[113] Targeting the fibrotic stiff stroma of tumors is another possibility. Even in tumors with cancer cells deficient in SMAD4 function, the epithelial cells contribute to stromal thickening through activation of other pathways, such as through JAK-mediated STAT3 signaling.[139] Mouse studies and patient tissues show that PDAC deficient in TGF-β signaling is more aggressive, in part because of EMT driven by a mechanically activated tumor microenvironment involving both TGF-β–responsive stromal cells and nonresponsive epithelial cells.[139]

Future Directions

Given the burden that these diseases and cancer have on the health care system (approximately $135.9 billion in 2015 for the United States) and the high mortality rates of many of these conditions, the ability to treat existing disease and prevent progression to severe disease or cancer is urgently needed.[119] Preclinical studies tested approaches to inhibiting TGF-β signaling: neutralizing antibodies against the TGF-β subfamily or the receptor, ligand traps, small molecule inhibitors of the kinase activity of the receptor or integrin-mediated activation of the ligand latent complexes or antisense oligonucleotides targeting TGF-β2. However, neutralizing antibodies and some TGF-β receptor inhibitors have resulted in cardiac toxicity in nonhuman primates, dogs, and rodents[140–142] and skin toxicity, including skin cancer in patients.[143] Although the TGFBR1 inhibitor galunisertib is

in clinical trials for CRC, HCC, and PDAC,[144,145] toxicities may preclude systemic targeting of TGF-β.

Targeted blocking of specific TGF-β signals could be beneficial. Inspired by high TGF-β signaling in CAFs of patients with urothelial cancer that failed to respond to immune checkpoint inhibition (the PD-L1 antibody atezolizumab), blocking stromal TGF-β signaling improves the response to a PD-L1–targeted antibody in a mouse model.[146] In a melanoma model, specifically blocking the TGF-β present on the surface of Tregs restores anti-tumor cytotoxicity mediated by effector T cells ex vivo.[147] Thus, targeted disruption of tumor-enabling signaling within the tumor microenvironment or combining TGF-β signaling inhibitors with immune-modulating therapies may be therapeutic options that overcome toxicities associated with systemic TGF-β inhibition.

Other options for limiting toxicity of TGF-β–targeted therapies include stratifying patients to treat only those with high signatures of activated TGF-β signaling. For example, patients with activated TGF-β signaling signatures that correlate with poor prognosis have been identified among patients with HCC,[107] PDAC,[148] and CRC.[149] Using treatment holidays to limit toxicity or combination with other therapies to reduce the effective dose are also possibilities. Targeting pathways that are aberrantly activated by disruption of TGF-β signaling or targeting regulators of TGF-β signaling, such as adaptors or E3 ubiquitin ligases, could yield safer alternatives. For example, targeting the TGF-β–regulated protein vascular endothelial growth factor and immune checkpoint blockade in HCC.[150]

Conclusions

Efforts ranging from analysis of patient tissue to development of mouse models to studies of cells in culture have yielded progress in understanding the complex roles of TGF-β signaling. Mouse models have been critical (Table 1, Supplementary Table 1). Given the complexity in cells that produce and respond to TGF-β signals, single-cell approaches will help determine the sources of TGF-β and the responding cells and provide insight regarding how specific cell types respond to these signals or adapt to the absence of the ability to respond to these signals and clarify the roles of TGF-β signaling in various stages of disease. Such information may pave the way for combination therapies and identify TGF-β pathway biomarkers heralding the switch from dysplasia to cancer, identifying high-risk individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper:

BE	Barrett's esophagus
BMP	bone morphogenetic protein
CAF	cancer-associated fibroblast
CRC	colorectal cancer
DC	dendritic cell
EAC	esophageal adenocarcinoma
EBV	Epstein-Barr virus
ECM	extracellular matrix
EMT	epithelial-to-mesenchymal transition
GC	gastric cancer
GI	gastrointestinal
IBD	inflammatory bowel disease
IL	interleukin
MMP	matrix metalloproteinase
MSI	microsatellite instability
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
PanIN	pancreatic intraepithelial neoplasia
PDAC	pancreatic ductal adenocarcinoma
TGF-β	transforming growth factor β
Th	T helper cell
TIMP	tissue inhibitor of metalloproteinases
Treg	regulatory T cells
UC	ulcerative colitis

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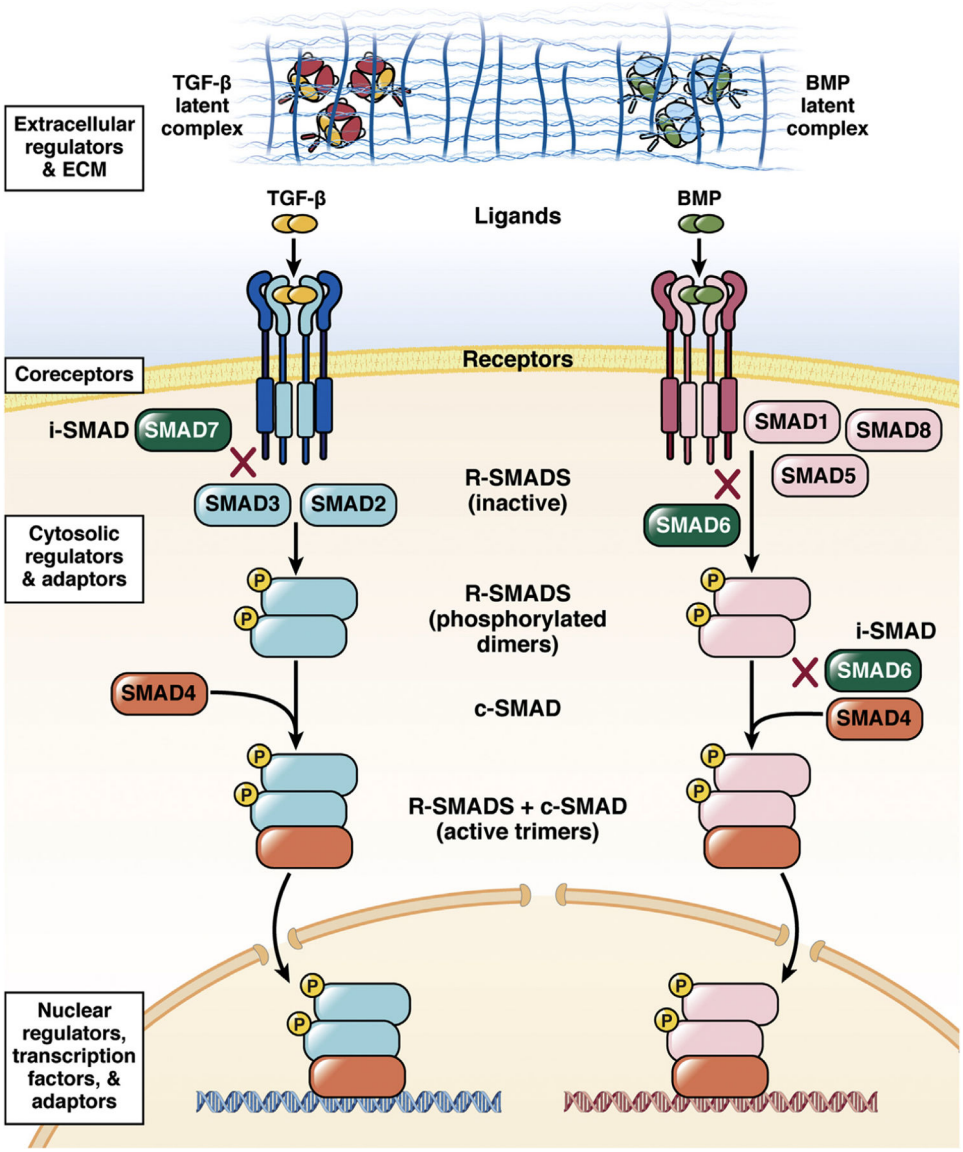

Figure 1.

TGF-β signaling pathways. A simplified view of the core of the TGF-β and BMP pathways. X indicates an inhibitory interaction. The ligands are retained as inactive procomplexes, often associated with the ECM, before activation. The classes of various regulators are indicated at each level of the pathways in white boxes.

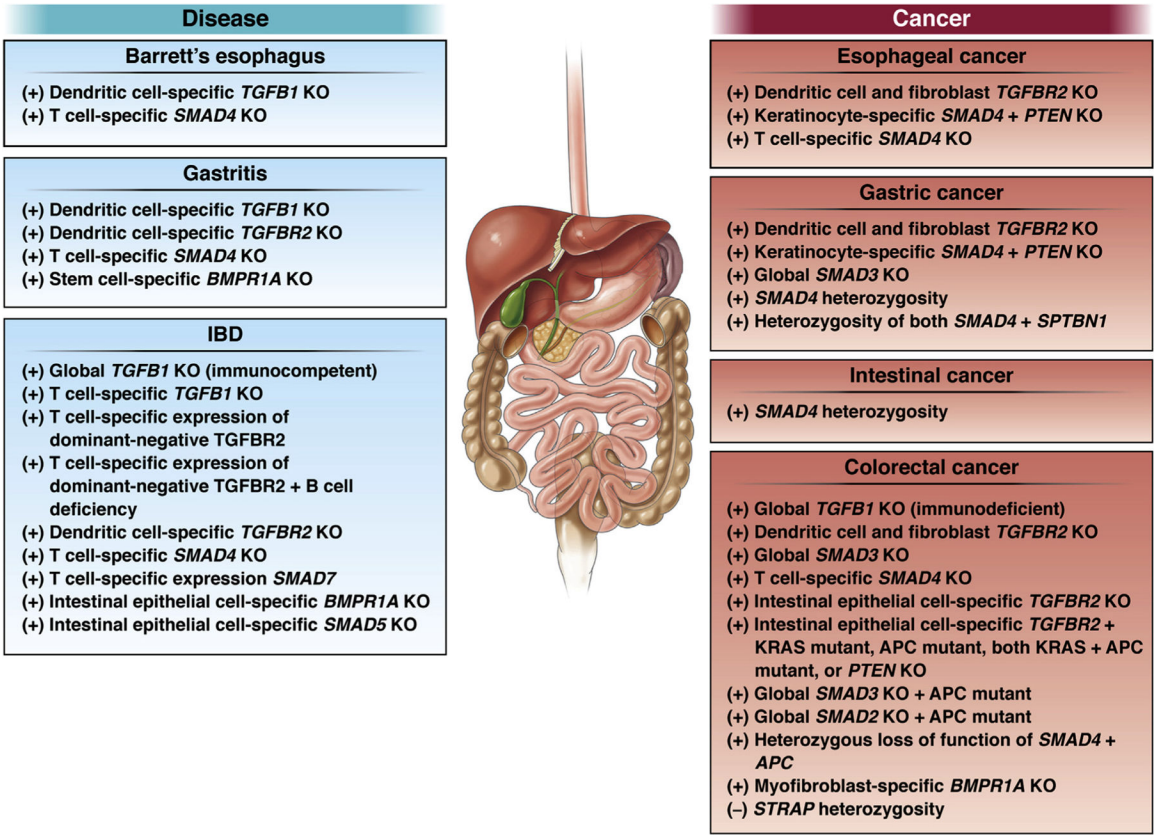

Figure 2.

GI tract with relevant mouse models for investigating TGF-β signaling in disease or cancer. (+), increased susceptibility to disease or cancer; (–), decreased susceptibility.

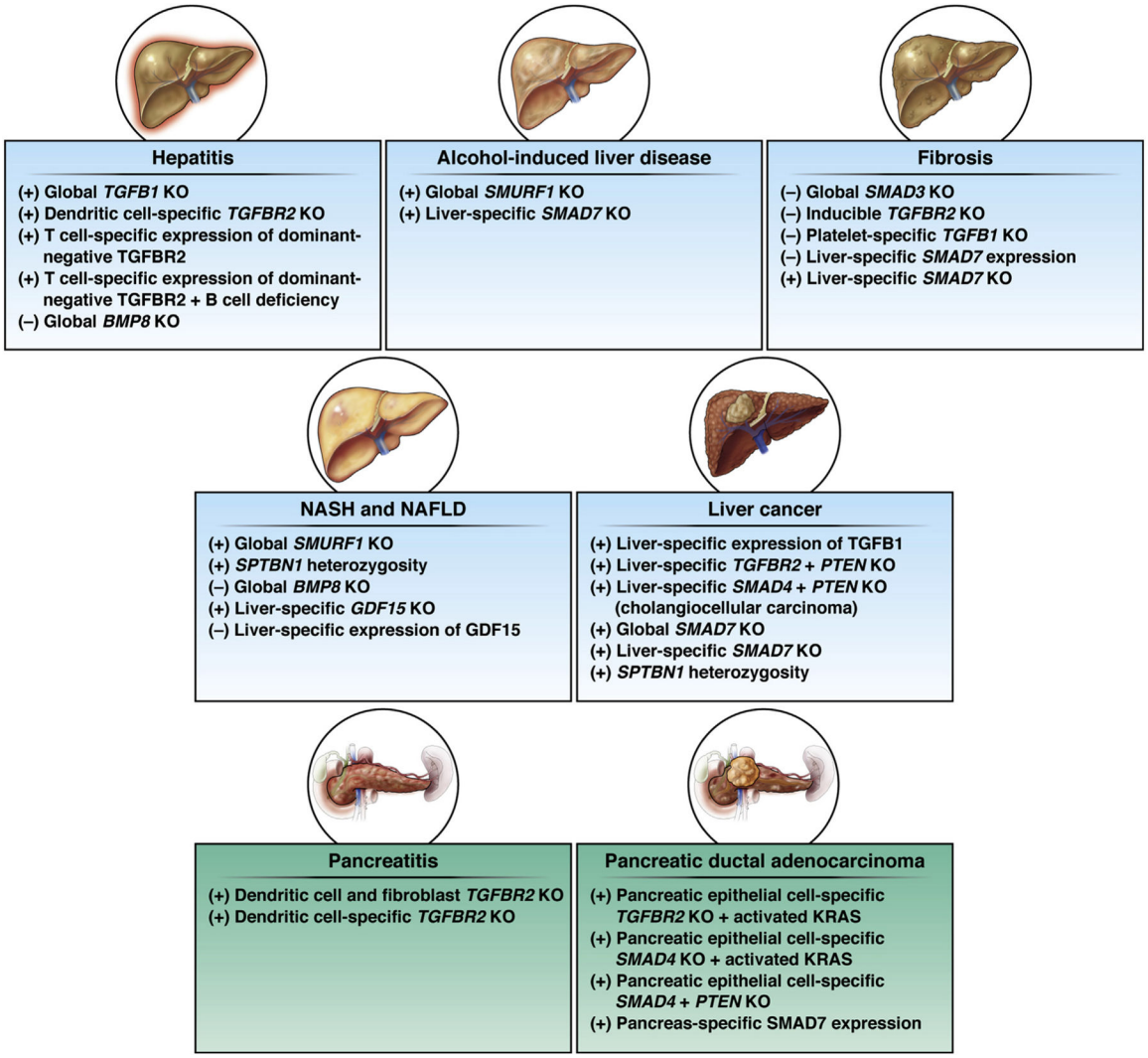

Figure 3.

Pancreas and liver with relevant mouse models for investigating TGF-β signaling in disease or cancer. (+), increased susceptibility to disease or cancer; (–), decreased susceptibility.

Table 1.

Mouse Models Mentioned in the Text for Studying the Roles of Transforming Growth Factor–β Signaling in Digestive Diseases and Cancers

Gene (and function)	Mice model	Disease relevance	Model design	Model phenotype	Reference no.
TGFB1 (ligand)	*Tgfb1*$^{+/-}$ TGFB1 heterozygosity (C57B1/6 NCr)	Liver cancer	Disruption of *TGFB1* exon 1 and intron 1 with neomycin-resistance cassette	By 6 mo, mice have increased susceptibility to chemically induced liver cancer.	96
	Tgfb1$^{-/-}$ Immunocompetent *TGFB1* knockout (C3H/HeN × C57BL/6J)	Inflammation, IBD	Disruption of *TGFB1* exon 6 with neomycin-resistance cassette	By 3 wk, mice die from a wasting syndrome with multifocal, mixed inflammatory cell response and tissue necrosis.	32
	*TGF-*β$^{\Delta DC}$ DC-specific *TGFB1* knockout (C57BL6)	Gastritis	Disruption of *TGFB1* exon 6 with cCD11c-Cre driving DC-specific knockout	After 6 mo of *Helicobacter felis* infection, mice develop severe gastritis, with a trend toward increased metaplasia.	17
	CD4-Cre/Tgfb1$^{fl/fl}$ T cell–specific *TGFB1* knockout (C57BL/6)	IBD	LoxP sites flanking *TGFB1* exon 1 with CD4-Cre driving T cell–specific knockout	By 4–12 mo, mice developed inflammatory disorder and severe colitis.	36
	Tgfb1$^{-/-}$*Rag2*$^{-/-}$ Immunodeficient *TGFB1* knockout (129S6 × CF-1)	CRC	Disruption of *TGFB1* exon 6 with neomycin-resistance cassette	By 5 mo, all mice develop severe hyperplasia and nonmetastatic carcinoma in the cecum and colon.	59
	PF4CreTgfb1$^{f/f}$ Platelet-specific *TGFB1* knockout (C57BL/6)	Liver fibrosis	LoxP sites flanking *TGFB1* exon 6, with PF4-Cre driving deletion in platelets	Mice develop less liver fibrosis in response to chemically induced liver damage.	83
	Alb/TGF-B1 Liver-specific expression of TGF-β1 (C57BL/6 × CBA/J)	Liver cancer	Porcine *TGFB1* expressed from *albumin* promoter	By 16–18 mo, ~60% of mice spontaneously develop HCC	101
	myc/TGF-B1 Liver-specific expression of MYC and TGF-β1 (C57BL/6 × CBA/J)	Liver cancer	*MYC* and porcine *TGFB1* expressed from *albumin* promoter	By 13 mo, 100% of mice develop multifocal tumors in different lobes of the liver.	101
	*Alb-TGF-*β*1/ LFABP-cyclin D1* Liver-specific expression of TGF-β1 and multi-tissue expression of cyclin D1 (B6CBA × C57BL/6)	Liver cancer	Porcine *TGFB1* expressed from albumin promoter and *CCND1* expressed from LFABP promoter	By 12 mo, 69% of mice develop liver cancer or high-grade tumors.	102
TGFBRII (Type II receptor subunit for TGFβ1, TGF-β2, TGF-β3)	*Tgfbr2*^{fspKO} *TGFBR2* knockout specifically in cells positive for S100A4 (DBA× Balb/c× C57BL/6)	Pancreatitis, esophageal and gastric cancer	LoxP sites flanking *TGFBR2* exon 2, with FSP1-Cre driving deletion in multiple cells of mesenchymal origin, including DCs and stromal fibroblasts	By 6 wk, mice spontaneously develop autoimmune pancreatitis. By 7 wk, all mice spontaneously develop invasive squamous cell carcinoma of the forestomach.	16
	CRP/ΔkTβRII Liver-specific expression of dominant-negative TGFBR2	Liver cancer	Dominant-negative TGFBR2 with CRP promoter driving liver-specific expression	Mice exhibit increased susceptibility to chemically induced multifocal preneoplastic lesions and liver cancer.	97
	CD4-dnTGFβRII T cell–specific expression of dominant-negative TGFBR2 (C57BL/6 × 6XC3H)	IBD, hepatitis	Dominant-negative TGFBR2 with *CD4* promoter driving T cell–specific expression	By 4 mo, mice spontaneously develop IBD; mice have increased susceptibility to chemically induced liver disease.	37

Gene (and function)	Mice model	Disease relevance	Model design	Model phenotype	Reference no.
	DC-*Tgfbr2* KO DC-specific *TGFBR2* knockout (B6.129S6)	Hepatitis, pancreatitis, colitis, gastritis	Flox sites flanking *TGFBR* exon2 with CD11c-Cre driving DC-specific deletion	By 15 wk, mice die of autoimmune inflammation in multiple organs of digestive tract.	38
	$Apc^{1638N/wt}Tgfbr2^{IEKO}$ Intestinal epithelial cell–specific *TGFBR2* knockout with heterozygous *APC* mutation (C57BL/6JIco × C57BL6)	CRC	LoxP sites flanking *TGFBR2* exon 2, with Villin-Cre driving intestinal epithelial cell–specific deletion in the context of heterozygous *APC* loss of function	By 12 mo, all mice spontaneously develop intestinal adenocarcinoma that progresses to invasive disease.	67
	$Apc^{\Delta 716}$ $Tgfbr2^{\Delta IEC}$ Intestinal epithelial cell–specific *TGFBR2* knockout with *APC mutation*	CRC	LoxP sites flanking TGFBR2 exon 2, with Villin-Cre driving intestinal epithelial cell–specific deletion in the context of APC mutation	By 15 wk, mice spontaneously develop intestinal adenocarcinomas with submucosal invasion in large polyps.	68
	LAKTP $Lgr5^{eGFPCreERT2}/Apc^{Loxp}/Kras^{LSL\_G12D}/Tgfbr2^{Loxp}/Trp53^{Loxp}$ Intestinal stem cell-specific knockout of *APC, TGFBR2, TRP53* with KRAS activation (C57BL/6J)	CRC	LoxP sites flanking regions of *APC*, *TGFBR2*, and *TRP53* with inducible with $Lgr5^{eGFP\text{-}creERT2}$ driving intestinal stem cell-specific deletion and expression of activating KRAS mutant	Mice exhibit increased susceptibility of chemically induced metastatic colon cancer.	71
	$Ptf1a^{cre/+}LSL\text{-}Kras^{G12D/+}Tgfbr2^{flox/flox}$ Pancreatic epithelial cell–specific *TGFBR2* knockout with KRAS activation (C57BL/6×DBA/2×129/SvJae)	Pancreatic cancer	LoxP sites flanking *TGFBR2* exon 2, with Ptf1a-Cre driving pancreatic epithelial cell–specific deletion and expression of activating KRAS mutant	By 2 mo, mice die of PDAC.	122
SMAD3 (R-SMAD activated by TGF-β1, TGF-β2, TGF-β3, Activin A, Activin B, Nodal, GDF1, GDF2, GDF8, GDF9, GDF11)	$Smad3^{-/-}$ *SMAD3* knockout (C57BL/6 × 129/S)	Gastric cancer, CRC	Disruption of *SMAD3* exon 2 with IRES-LacZ and neomycin cassette	By 10 mo, mice spontaneously develop invasive gastric cancer arising in the forestomach–stomach junction. By 4–6 mo, mice (30%) spontaneously develop large polyps and aggressive, metastatic colon cancer that depends on *Helicobacter*.	60
	$Smad3^{ex8/ex8}$ *SMAD3* knockout (C57BL/6 × Black Swiss)	IBD	Targeted deletion of *Smad3* exon 8 by homologous recombination, resulting in disruption of the interaction between SMAD3 and the *TGFB* receptor	More than 6 mo, nearly 77% of the mice spontaneously developed chronic inflammation in the intestines.	31
	$Smad3^{-/-}$ + *Helicobacter* *SMAD3* knockout infected with *Helicobacter* (129/J)	CRC	Disruption of *SMAD3* exon 2 with IRES-LacZ and neomycin cassette	Mice exhibit increased susceptibility to infection-induced colon cancer.	60, 61
	$Apc^{Min/+}Smad3^{-/-}SMAD3$ knockout with heterozygous *APC* mutation (129/Sv)	CRC	Disruption of *SMAD3* exon 2 with neomycin cassette and mutation of *APC*	By 2 mo, mice spontaneously develop tumors in the distal colon, resembling human familial adenomatous polyposis.	70
SMAD4 (c-SMAD)	$Smad4^{co/co;\ Lck\text{-}cre}$ $Smad4^{co/co;\ CD4\text{-}cre}$ T cell–specific *SMAD4* knockout (C57BL/6 × SvEv129 × FVB)	Gastrointestinal epithelial cancer	LoxP sites flanking *SMAD4* exon 8, with Lck-Cre or CD4-Cre driving T cell–specific deletion	Mice spontaneously develop inflammation and epithelial cancers throughout the GI tract.	65

Gene (and function)	Mice model	Disease relevance	Model design	Model phenotype	Reference no.
	Smad4$^{+/E6sad}$ *SMAD4* heterozygosity (129Ola × C57BL/6JIco)	Intestinal cancer	Single nucleotide deletion of *SMAD4* exon 6	By 9–18 mo, mice spontaneously develop adenomas and mixed polyposis of the upper GI tract.	69
	Apc$^{+/1638N}$/*Smad4*$^{+/E6sad}$ Heterozygous loss of function of both *SMAD4* and *APC* in cis or trans (129Ola × C57BL/6JIco)	CRC	Single nucleotide deletion of *SMAD4* exon 6, and disruption of *APC* exon 15	Both trans and cis mice spontaneously develop tumors of the GI tract, desmoids, and epidermal tumors. Trans mice spontaneously develop high numbers of tumors. Cis mice spontaneously develop rapidly progressing disease, dying within 6 wk.	69
SMAD7 (I-SMAD for SMAD2 and SMAD3)	*Smad7Tg* T cell–specific expression of *SMAD7* (C57BL/6)	IBD	SMAD7 with CD2 promoter/enhancer driving T cell–specific expression	Mice exhibit more susceptibility to chemically induced colitis but fewer colitis-induced tumors.	77
	S7tg Hepatocyte-specific expression of SMAD7 (C57BL/6)	Liver fibrosis	Flag-tagged SMAD7 with CRP promoter driving hepatocyte-specific expression	Mice exhibit decreased susceptibility to chemically induced liver damage and fibrosis.	108
	Smad7 KO *SMAD7* knockout (C57BL/6)	Liver cancer	Disruption of *SMAD7* exon 1 with PGKneobpA cassette	Mice exhibit increased susceptibility to chemically induced HCC.	110
	TTR-Cre-SMAD7 KO, Hepatocyte-specific *SMAD7* knockout (C57BL/6)	Liver cancer	LoxP sites flanking SMAD7 exon 1 with TTR-Cre driving hepatocyte-specific deletion	Mice exhibit increased susceptibility to chemically induced HCC.	109
	SMAD7Tg Pancreas-specific expression of SMAD7 (DBA2)	Pancreatic cancer	Myc-tagged SMAD7 with elastase I promoter driving pancreas-specific expression	By 6 mo, mice develop PanIN.	126
SPTBN1 (Adaptor for activated SMAD2 and SMAD3)	*SMAD7Tg* Pancreas-specific expression of SMAD7 (DBA2) *Smad4*$^{+/-}$ *Sptbn1*$^{+/-}$Heterozygous loss of function of both *SMAD4* and *SPTBN1* (129SvEv × C57BL/6)	Gastric cancer, liver cancer	Disruption of *SPTBN1* exon 25 and *SMAD4* exon 8 with neomycin cassette	All mice spontaneously develop gastric polyps with a subset progressing to cancer; a subset of mice develop colon cancer.	62, 64
	Sptbn1$^{+/-}$ *SPTBN1* heterozygosity (129SvEv/Black Swiss)	Liver steatosis, fibrosis, liver cancer	Disruption of *SPTBN1* exon 25 with neomycin cassette	By 15 mo, 40% of mice spontaneously develop liver diseases and cancer.	99
	Sptbn1$^{+/-}$Iitih4$^{-/-}$ Heterozygous loss of *SPTBN1* and knockout of *ITIH4*	Liver cancer	Disruption of *SPTBN1* exon 25, and *ITIH4* exons 2 and 3 with neomycin cassette	Mice exhibit increased susceptibility to liver cancer.	100

c-SMAD, common SMAD; I-SMAD, inhibitory SMAD; R-SMAD, receptor-activated SMAD.