Bone Morphogenetic Protein Signaling in Liver and Gastrointestinal Diseases and Cancer

Multiple receptor complexes respond to BMPs, but all of the BMPs signal through SMAD1 and SMAD5. Although the TGF-β and BMP families signal through distinct receptor complexes and through distinct SMAD complexes, the pathways intersect at SMAD4, SMAD6, and SMAD7,[1,2] and these pathways can have cooperative or counteracting effects through the genes that they regulate.

Bone Morphogenetic Proteins in Barrett's Esophagus and Esophageal Cancer

BMP4 and BMP5 have also been implicated in BE and EAC. Patients with BE have significantly increased plasma concentrations of BMP5, which may serve as a blood biomarker for screening high-risk BE populations.[3] BE and EAC tissue have increased BMP4 signaling and studies with cultured BE and EAC cell lines suggest that this pathway promotes EMT by induction of *SNAIL2*.[4]

Bone Morphogenetic Proteins in Gastritis, Infection, and Gastric Cancer

Studies in mice indicate that impaired BMP4 signaling in parietal cells and LGR5-positive stem cells stimulates epithelial stem cells, epithelial remodeling, and metaplasia in response to inflammation triggered by *H pylori* infection.[5,6] Studies with mice and cultured cells show that BMP4 signaling in gastric epithelial cells inhibits the release of inflammatory mediators and reduces inflammation.[7]

Alterations in BMP signaling are associated with metastasis of GC. BMP4 is frequently overexpressed in GC both at the messenger RNA and protein levels; high amounts of BMP4 correlate with poor prognosis.[8] Mechanistically, BMP4 promotes EMT and invasive properties of GC cells. Studies with cultured endothelial cells show that suppression of autocrine TGF-β signaling in endothelial cells by the transcription factor GATA6 increases endothelial cell survival and angiogenic activity by shifting the balance of signals from those mediated by SMAD2 and SMAD3, activated by the TGF-β subfamily, to those mediated by SMAD1 and SMAD5, activated by BMP9 and BMP10.[9]

Amplification of *GATA6*, *GATA4*, or *KLF5* is common in GC, and amplification of 1 is sufficient to induce the expression of the other 2, thereby promoting tumorigenesis.[10] A mouse study connected the complex formed by these 3 transcription factors to a feed-forward differentiation mechanism activated by multiple BMPs (BMP1, BMP2, and BMP8).[11] Whether disruption of this pathway contributes to GC is unknown. Lymph node metastasis of GC is significantly associated with high amounts of phosphorylated SMAD2.[12]

Bone Morphogenetic Proteins in Hereditary Colon Cancer Syndromes

Many cases of CRC have a strong hereditary component; most have mutations that hyperactivate Wnt/β-catenin signaling, such as mutations in *APC*. However, mutations in *BMPR1A* and *SMAD4* cause familial juvenile polyposis syndrome, which is primarily a disease characterized by benign gastric and intestinal polyps. Both patients with and without these mutations and who have disease-causing mutations in either *BMPR1A* or *SMAD4* had increased risk of developing GI adenomas.[13] Mice with stromal-specific knockout of *BMPR2* develop intestinal polyps that are associated with increased epithelial cell proliferation and reduced apoptosis and that have increased numbers of myofibroblasts in the lamina propria. However, these mice do not spontaneously develop adenocarcinomas.[14] Other hereditary forms of CRC include individuals with Lynch syndrome, which is also known as hereditary nonpolyposis CRC. CRC in individuals with Lynch syndrome have the CSM3 type CRC; this syndrome results from autosomal-dominant germline mutations in genes encoding proteins involved in DNA mismatch repair MLH1, MSH2, MSH6, and PMS2.[15] Mutations or changes in gene expression that co-occur in CRC of patients with Lynch syndrome or MSI include *ACVR2* (encoding a type II receptor for BMPs),[16] *TGFBR2*,[16–18] and *TGFBR1*.[19] Thus, altered signaling by BMPs or the TGF-β subfamily is likely a contributing factor in the progression to cancer.

Bone Morphogenetic Proteins in Nonalcoholic Fatty Liver Disease, Nonalcoholic Steatohepatitis, and Hepatocellular Carcinoma

Only a few BMPs have been investigated in relation to NAFLD, NASH, and HCC. In a mouse model of NAFLD, virus-mediated BMP4 overexpression in the liver reduced hepatic steatosis by inhibiting translation and stimulating lipid turnover in hepatocytes.[20] In contrast, BMP8, which increases with disease progression in mice and patients, contributes to hepatic stellate cell activation and limits NASH progression in mice.[21] In HCC, tumors with low *BMP10* expression are associated with poorer survival.[22] BMP9 is produced by hepatocytes.[23] A major role for BMP9 in the liver that is relevant for HCC is as a stimulator of angiogenesis.[24] Indeed, antibodies or antibody-based fusion proteins that block BMP9 binding to the receptor ALK1 (encoded by *ACVRL1*) or the co-receptor endoglin have anti-angiogenic effects,[25] and TRC105 and dalantercept are 2 such antibodies tested in clinical trials for HCC.[26,27]

Transforming Growth Factor–β Signaling in Celiac Disease

Celiac disease (CE) is a gluten-induced chronic small intestinal enteropathy.[28,29] Under normal conditions, the small intestine is a site of high concentrations of TGF-β, which suppress Th1 differentiation, promote Treg differentiation,[30] and stimulate IgA production.[31–33] These TGF-β–dependent immune responses prevent inflammation-induced intestinal damage (Figure 2). The effect of TGF-β on T cells depends on both the concentration of this cytokine and the presence of other signals. In the presence of IL-6, TGF-β induces Th17 differentiation at the expense of Treg differentiation[34]; without IL-6, TGF-β

diverts differentiation toward Treg cells.[30-35] CE is associated with a mixture of pro-inflammatory and immunosuppressive cytokines. Many of the pro-inflammatory cytokines influence the immune cell response to TGF-β. Serum concentrations of signals that intersect with the TGF-β pathway, including IL-6, tumor necrosis factor–α (TNF-α), interferon (IFN)-gamma, and IL-15, are either increased in patients with active CE[36] or upon gluten challenge.[37]

In CE, expansion of T cell populations that react to peptides from the cereal proteins in the gliadin family occurs, resulting in an inflammatory Th1 response that includes production of IFN-gamma and IL-21, as well as IL-4, which is associated with Th2 cells.[38,39] Contradictorily, increased transcripts for genes encoding IL-10 and TGF-β1 are present in both the surface epithelium and lamina propria,[40] and these signals should limit the inflammatory Th1 response.[41] Similarly, the lamina propria of the intestine of patients with CE has high amounts of innate inflammatory mediators IL-15, TNF-α, and IFN-gamma,[42,43] all of which impair the immunosuppressive effects of TGF-β.[41,44,45] IFN-gamma induces the expression of *SMAD7*,[45] and both TNF-α and IL-15 act through the Jun transcription factors to interfere with SMAD3-dependent gene regulation.[41,44] This apparent paradoxical milieu of both pro-inflammatory and immunosuppressive cytokines may reflect the body's attempt to counterbalance the abnormal immune activation that occurs in CE. Although patients with CE have a 14-fold higher risk of developing small bowel adenocarcinoma,[46,47] this represents <1% of GI cancers in the United States.

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Supplementary Table 1. Comprehensive List of Mouse Models for Studying the Roles of Transforming Growth Factor–β and Bone Morphogenetic Protein 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, 48
	Tgfb1$^{-/-}$ Immunocompetent *TGFB1* knockout (Balb/c)	Hepatitis	Disruption of *TGFB1* exon 6 with neomycin-resistance cassette	Mice spontaneously develop IFN-gamma–dependent necroinflammation of the liver.	49, 50
	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, 51
	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-β1/ 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

Supplementary Table 1. Continued

Gene (and function)	Mice model	Disease relevance	Model design	Model phenotype	Reference no.
TGFBRII (Type II receptor subunit for TGF-β1, TGF-β2, TGF-β3)	*Tgfbr2*^{fspKO} *TGFBR2* knockout specifically in cells positive for S100A4 (DBA× Balb/c× C57BL/6)	Pancreatitis, esophageal and GC	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, 52
	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, 53
	Igμ$^{-/-}$*dnTGFβRII* T cell–specific expression of dominant-negative TGFBR2 with B cell deficiency (C57BL/6×129S2)	IBD, biliary cirrhosis	Dominant-negative TGFBR2 with *CD4* promoter driving T cell–specific expression with *Igmu* knockout eliminating B cells	By 5 mo, mice spontaneously develop bile duct injury and necrosis, as well as severe colitis.	54
	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 develop 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, 55, 56
	Apc^{Δ716} *Tgfbr2*^{ΔIEC} 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

Supplementary Table 1. Continued

Gene (and function)	Mice model	Disease relevance	Model design	Model phenotype	Reference no.
	$Apc^{\Delta 716}$ $Kras^{G12D}$ $Tgfbr2^{IEKO}$ Intestinal epithelial cell–specific *TGFBR2* knockout with *APC* mutation and KRAS activation (C57BL/6)	CRC	LoxP sites flanking *TGFBR2* exon 2, with Villin-Cre driving intestinal epithelial cell–specific deletion and expression of activating *KRAS* mutation in the context of *APC* mutation	By 13–16 wk, mice spontaneously develop intestinal adenocarcinomas that progressed to invasive disease.	55, 57, 58
	$Tgfbr2^{IEKO}$;LSL-$Kras^{G12D/wt}$ Intestinal epithelial cell–specific *TGFBR2* knockout with KRAS activation (C57BL/6)	CRC	LoxP sites flanking *TGFBR2* exon 2, with Villin-Cre driving intestinal epithelial cell–specific deletion and expression of activating KRAS mutant	By 22 wk, 70% of mice spontaneously develop intestinal adenocarcinomas with a subset developing metastatic disease.	55, 59
	LAKTP $Lgr5^{eGFPCreERT2}$/Apc^{Loxp}/$Kras^{LSL\text{-}G12D}$/$Tgfbr2^{Loxp}$/$Trp53^{Loxp}$ 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
	$Pten^{IEKO}Tgfbr2^{IEKO}$ Intestinal epithelial cell–specific *TGFBR2* and *PTEN* knockout (C57BL/6J)	CRC	LoxP sites flanking *TGFBR2* exon 2 and *PTEN* exon5, Villin-Cre driving intestinal epithelial cell–specific deletion	By 54 wk of age, 86% of mice spontaneously develop intestinal adenocarcinomas with a subset developing metastatic disease.	55, 60, 61
	$Fabpl^{4xat\text{-}132}$ Cre $Tgfbr2^{flx/flx}$ Intestinal epithelial cell–specific *TGFBR2* deletion during embryogenesis	CRC	LoxP sites flanking *TGFBR2* exon 2, Fabp1-Cre drives intestine-specific deletion during embryogenesis	Mice have increased susceptibility to chemically induced colon cancer.	55, 62, 63
	TGF-βIIR(fl/fl)/Mx-Cre^{+} Inducible *TGFBR2* knockout (129/Sv x C57BL/6J)	Liver fibrosis	LoxP sites flanking *TGFBR2* exon 2, with Mx-Cre driving deletion induced by polyinosinic–polycytidic acid injection	Mice have decreased susceptibility to chemically induced liver fibrosis.	55, 64, 65
	$Pten^{LKO}Tgfbr2^{LKO}$ Liver-specific deletion of *PTEN* and *TGFBR2* knockout (C57BL6 × 129)	Liver cancer	LoxP sites flanking *TGFBR2* exon 2 and *PTEN* exon 5, with Alb-Cre driving liver-specific deletion	By 14 mo, ~86% of mice spontaneously develop liver tumors.	66, 67

Supplementary Table 1. Continued

Gene (and function)	Mice model	Disease relevance	Model design	Model phenotype	Reference no.
	Ptf1a$^{cre/+}$*LSL-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, 68
BMP8B (ligand)	BMP8B-KO *BMP8B* knockout (C57Bl6/J)	Hepatitis, NASH	Disruption of *BMP8B* exon 4 with MClneo cassette	Mice exhibit decreased susceptibility to chemically induced liver injury and diet-induced NASH.	69, 70
BMPR1 (type 1 receptor subunit for BMP2, BMP4, BMP5, BMP6, BMP7, BMP8, GDF5, GDF6, GDF7, AMH)	*Villin-Cre;Bmpr1a*$^{flox/flox}$ Intestinal epithelial cell–specific *BMPR1A* knockout (C57BL/6)	IBD	LoxP sites flanking *BMPR1A* exon 2, with Villin-Cre driving intestinal epithelial cell–specific deletion	Mice exhibit increased susceptibility to chemically induced colon injury and inflammation.	71, 72
	Bmpr1a$^{\Delta MES}$ Stomach and intestinal mesenchymal cell–specific *BMPR1A* knockout (C57BL/6J)	CRC	LoxP sites flanking *BMPR1A* exon 2, with Foxl1-Cre driving myofibroblast-specific deletion in mesenchymal cells of the stomach and intestine	By 12 mo, mice spontaneously develop polyps in the colon with increased numbers of fibroblasts and myofibroblasts.	72, 73
	Bmpr1a/Mx1-Cre Inducible *BMPR1A* knockout (C57BL/6)	Juvenile polyposis syndrome	LoxP sites flanking *BMPR1A* exon 2, Mx1-Cre driving deletion induced by polyinosinic–polycytidic acid injection.	Mice spontaneously develop intestinal polyposis.	74, 75
	Lgr5-Cre;Bmpr1a$^{flox/flox}$ Stem cell (LGR5^{+})–specific *BMPR1A* knockout (C57BL/6)	Gastric inflammation	LoxP sites flanking *BMPR1A* exon 2, Lgr5-Cre driving stem cell–specific deletion	Mice exhibit increased susceptibility to infection-induced gastric inflammation and development of dysplasia.	72, 75
SMAD2 (R-SMAD activated by TGF-β1, TGF-β2, TGF-β3, Activin A, Activin B, Nodal, GDF1, GDF2, GDF8, GDF9, GDF11))	*S2HeKO, S3KO* Liver-specific *SMAD2* knockout with global *SMAD3* knockout (C57BL/6)	Hepatitis	LoxP sites flanking *SMAD2* exon 2, with Alb-Cre driving liver-specific deletion and with disruption of *SMAD3* exon 8	Mice exhibit increased susceptibility to chemically induced liver injury.	76, 77
	Apc^{580D}*/Smad2* Heterozygous loss of function of both *SMAD2* and *APC* in cis (C57BL6/J)	CRC	Disruption of *SMAD2* exons 3 and 4 by PGK-neo cassette; LoxP sites flanking *APC* exon 14, with Cre driving deletion	By 30 wk, mice spontaneously develop intestinal polyps and tumors with rapid progression to malignancy.	78, 79

Supplementary Table 1. Continued

Gene (and function)	Mice model	Disease relevance	Model design	Model phenotype	Reference no.
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)	GC, CRC	Disruption of *SMAD3* exon 2 with IRES-LacZ and neomycin cassette	By 10 mo, mice spontaneously develop invasive GC 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, 80
	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
	Smad3$^{-/-}$*SMAD3* knockout (Black Swiss × 129SVJ)	Liver fibrosis	Disruption of *SMAD3* exon 8 with neomycin cassette	Mice are less susceptible to chemically induced liver fibrosis.	81
	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, 82, 83
SMAD5 (R-SMAD for BMP2, BMP4, BMP5, BMP6, BMP7, BMP8, BMP9, BMP10, BMP15, GDF5, GDF6, GDF7, AMH	*Smad5*$^{\Delta IEC}$ Intestinal epithelial cell–specific *SMAD5* knockout (C57BL/6)	IBD	LoxP sites flanking *SMAD5* exon 2, with Villin-Cre drives intestinal epithelial cell–specific deletion	Mice exhibit increased susceptibility to chemically induced colitis.	84, 85
SMAD4 (c-SMAD)	*Smad4*$^{Co/Co}$*; PTEN*$^{Co/Co}$*;K5-Cre* Keratinocyte-specific knockout of both *SMAD4* and *PTEN* (C57BL/6)	Esophageal and GC	LoxP sites flanking *SMAD4* exons 8 and 9 and *PTEN* exon 5 with keratin 5-Cre driving keratinocyte-specific deletion	By 2 mo, mice spontaneously develop invasive squamous cell carcinoma in the forestomach and stomach.	86–88
	Smad4$^{(8+/-)}$ *SMAD4* heterozygosity (129 × Black Swiss or 129 × C57B6)	GC	Disruption of *SMAD4* exon 8 with neomycin cassette	By 6–12 mo, mice spontaneously develop polyposis in the stomach that progresses to invasive cancer.	89–91

Supplementary Table 1. Continued

Gene (and function)	Mice model	Disease relevance	Model design	Model phenotype	Reference no.
	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
	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, 92
	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, 93
	cis-Apc$^{+/\Delta 716}$*Smad4*$^{+/-}$ Heterozygous loss of function of both *SMAD4* and *APC* in cis (129/Sv × C57BL/6)	CRC	Disruption of *SMAD4* exon 1 and *APC* exon 15 with neomycin cassette	Mice spontaneously develop polyps in large and small intestine that progress to invasive cancer.	58, 94, 95
	Smad4$^{Co/Co}$*Pten*$^{Co/Co}$*Alb-Cre* Liver-specific *SMAD4* and *PTEN* (C57BL/6)	Cholangiocellular carcinoma	LoxP sites flanking *SMAD4* exon 8 and *PTEN* exon 5, with Alb-Cre driving liver-specific deletion	By 4–7 mo, mice spontaneously develop cholangiocarcinoma.	87, 96
	Pdx1Cre; Kras$^{G12D/+}$*; Smad4*$^{lox/lox}$ Pancreatic epithelial cell–specific *SMAD4* knockout with KRAS activation (C57BL/6 × 129SvJae)	Pancreatic cancer	LoxP sites flanking *SMAD4* exon 8, with Pdx-Cre driving pancreatic epithelial cell–specific deletion and expression of activating KRAS mutant	Mice spontaneously develop premalignant PanIN that progresses to early-stage PDAC.	87, 97, 98
	Smad4$^{Co/Co}$*;Pten*$^{Co/Co}$*;Pdx-Cre* Pancreatic epithelial cell–specific *SMAD4* and *PTEN* knockout (129 × FVB × Black Swiss)	Pancreatic cancer	LoxP sites flanking *SMAD* 4 exon 8 and *PTEN* exon 5, with Pdx1-Cre driving pancreatic epithelial cell–specific deletion	By 0.5–4 mo, mice spontaneously develop ductal tumors that progress to PDAC with a subset developing metastatic disease.	87, 99

Supplementary Table 1. Continued

Gene (and function)	Mice model	Disease relevance	Model design	Model phenotype	Reference no.
SMAD7 (I-SMAD for SMAD2 and SMAD3)	*Smad7*$^{liver\text{-}KO}$ Liver-specific *SMAD7* knockout (C57BL/6)	Hepatitis; alcohol-induced liver injury	LoxP sites flanking *SMAD7* exon 4, with Alb-Cre driving liver-specific deletion	Mice (30%) spontaneously develop liver damage and all exhibit increased susceptibility to alcohol-induced liver injury and steatosis.	100
	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.	101, 102
	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, 103
	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, 104
	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.	128
SPTBN1 (adaptor for activated SMAD2 and SMAD3)	*Smad4*$^{+/-}$*Sptbn1*$^{+/-}$Heterozygous loss of function of both *SMAD4* and *SPTBN1* (129SvEv × C57BL/6)	GC, 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 ,89–e91
	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, 105
	Sptbn1$^{+/-}$*Itih4*$^{-/-}$ 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
SMURF1 (ubiquitin ligase that targets TGFBRI/TGFBRII and SMAD2 and SMAD3 to inhibit pathway)	*Smurf1*$^{-/-}$*SMURF1* knockout (C57BL/6)	NAFLD, alcohol-induced liver disease	Disruption of *SMURF1* exons 6, 7, and 8 with neomycin cassette	Mice exhibit increased susceptibility to alcohol-induced steatohepatitis and high-fat diet–induced liver disease.	106

Supplementary Table 1. Continued

Gene (and function)	Mice model	Disease relevance	Model design	Model phenotype	Reference no.
	Smurf1$^{-/-}$*SMURF1* knockout (Black Swiss × 129/SvEv)	NASH	Disruption of *SMURF1* exons 6, 7, and 8 with neomycin cassette	Old mice spontaneously develop NASH.	107
GDF15 (ligand)	*GDF15-Tg* Liver-specific *GDF15* expression (C57BL/6)	NASH	Liver-specific expression of human GDF15 from the pLiv7 vector	Mice exhibit reduced susceptibility to diet-induced NASH.	108
	Gdf15$^{-/-}$ *Gdf15* knockout mice (C57BL/6/ × 129/SvJ)	NASH, liver cancer	Disruption of *GDF15* exon 2 with neomycin cassette	Mice exhibit increased susceptibility to diet-induced NASH and increased susceptibility to chemically induced HCC.	108–110
STRAP (inhibitor of TGFBRI/TGFBRII signaling)	*Strap*$^{+/-}$ *STRAP* heterozygosity (C57BL/6)	CRC	Disruption of *STRAP* exon 3 and 4	Mice exhibit reduced susceptibility to chemically induced colon cancer that is associated with few cancer stem cells.	111, 112

NOTE. References for the original development of the mouse models and references for studies showing the phenotypes are included.
c-SMAD, common SMAD; I-SMAD, inhibitory SMAD; R-SMAD, receptor-activated SMAD.

Supplementary Table 2. Reviews Related to Transforming Growth Factor–β Signaling or Diseases and Cancers of the Gastrointestinal System

Review topic	Reference no.
TGF-β signaling	e113–e120
BE and esophageal cancer	e121–e123
IBD, hereditary colon cancer syndromes, and CRC	e124–e129
NAFLD, NASH, and HCC	93, e130–e135
Pancreatic cancer	e136–e138