Chapter 6
TGF-β as Tumor Suppressor: Lessons from Mouse Models

Ulrich Valcourt, David F. Vincent, and Laurent Bartholin

Abstract Transforming growth factor-β (TGF-β) signals through serine/threonine-kinase receptors to activate several intracellular signaling pathways, including the "canonical" Smad signaling pathway. TGF-β has tumor-suppressive functions through its capacity to induce growth arrest and apoptosis. Escape from the anti-proliferative effects of TGF-β is a hallmark of almost all tumors of epithelial origin. This chapter first depicts the genetic alterations ablating the TGF-β tumor-suppressive functions in human tumors. Then, the chapter presents the genetically engineered mouse models that have been developed by introducing genetic alterations found in humans. These mouse models demonstrated the tumor-suppressive role of TGF-β in vivo shedding light on its intricate relationship with the tumor microenvironment. In all, the presented mouse models of cancer with an impaired TGF-β signaling pathway provide an integrated view of the complex tumor-suppressive role of TGF-β and represent valuable tools for preclinical studies.

Keywords Cre/lox • Digestive tract • Epithelial-to-mesenchymal transition (EMT) • Homotypic cell cannibalism (HoCC) • Mammary gland • Mutations • Pancreas • Smad • Transgenic mice • Xenograft

Abbreviations

ALK5	Activin receptor-like kinase 5
AOM	Azoxymethane
APC	Adenomatous polyposis coli

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BMP	Bone morphogenetic protein
BMPR1A	Bone morphogenetic protein receptor, type IA
CTGF	Connective tissue growth factor
DEN	Diethylnitrosamine
DMBA	7,12-Dimethylbenz(α)anthracene
DPC4	Deleted in pancreatic carcinoma 4
ELF	Embryonic liver fodrin
EMT	Epithelial-to-mesenchymal transition
FAP	Familial adenomatous polyposis
HoCC	Homotypic cell cannibalism
IPMN	Intraductal papillary mucinous neoplasms
KSSTT	Pdx1-Cre; $KRAS^{G12D}$; Smad4-$KO^{L/L}$; TIF1γ-$KO^{L/L}$
LOH	Loss of heterozygosity
LRP	Low-density lipoprotein receptor-related protein
LSL	Lox-stop-lox
MAPK	Mitogen-activated protein kinase
MCN	Mucinous cystic neoplasms
Min	Multiple intestinal neoplasia
MMP	Matrix metalloproteinase
MMTV	Mouse mammary tumor virus
MSI	Microsatellite instability
PanIN	Pancreatic intraepithelial neoplasms
PDAC	Pancreatic ductal adenocarcinoma
PDGF	Platelet-derived growth factor
PyVmT	Polyoma virus middle T antigen
SCC	Squamous cell carcinoma
TGF-β	Transforming growth factor β
TGF-α	Transforming growth factor α
TIF1	Transcriptional intermediary factor 1
TPA	12-O-tetradecanoylphorbol-13-acetate
TβRI	TGF-β type I receptor
TβRII	TGF-β type II receptor
WAP	Whey acidic protein

6.1 Introduction: TGF-B Signaling and Genetic Alterations in Human Tumors

Transforming growth factor-β (TGF-β) is a secreted polypeptide belonging to a large family of cytokines and growth factors including TGF-βs, bone morphogenetic proteins (BMPs), and activins. The three isoforms of TGF-β (TGF-β1, TGF-β2, and TGF-β3) are secreted to different levels by most cell types, and they act as positive and negative regulators of differentiation and proliferative programs

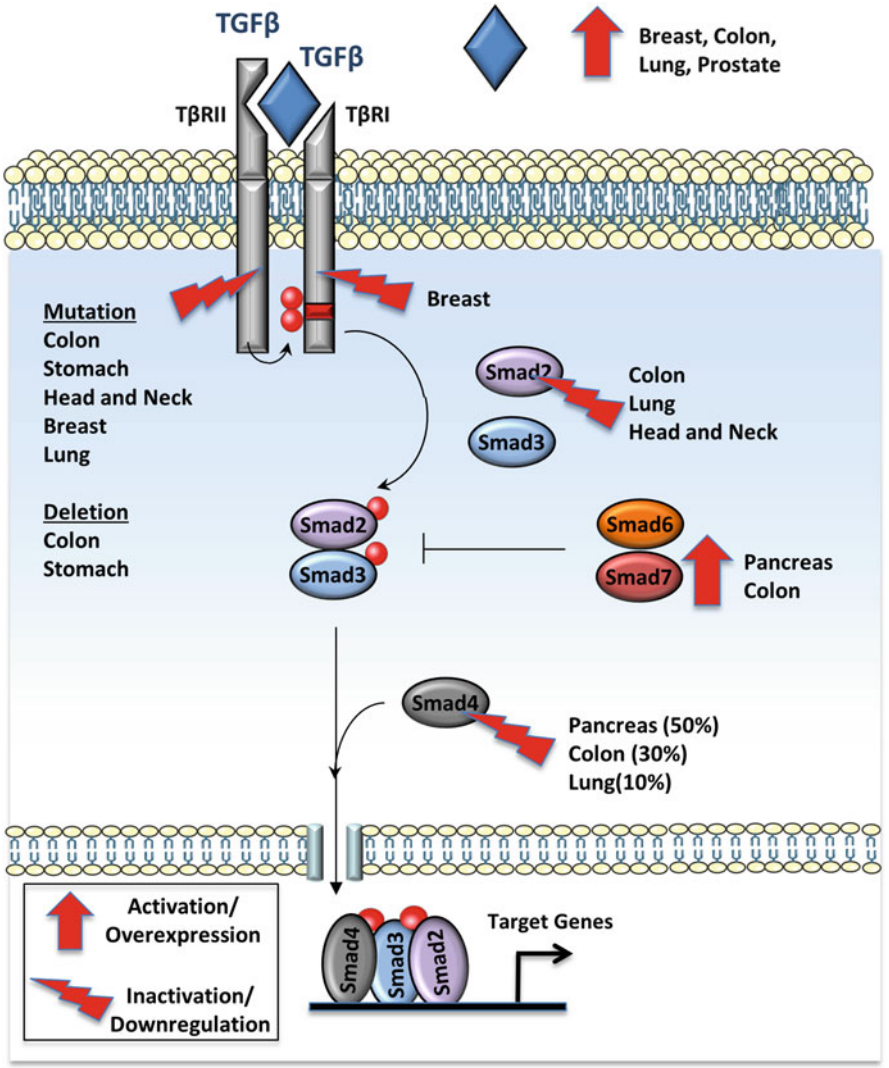

Fig. 6.1 Genetic alterations in human tumors affecting TGF-β signaling

(Letterio and Roberts 1998). TGF-βs play a crucial role in physiological processes involved in embryonic development, immune responses, cell growth control, and wound healing. All three TGF-β isoforms signal through a common heterotetrameric serine/threonine-kinase receptor complex (Massague 1992) comprising TβRII and TβRI (also termed activin receptor-like kinase 5, ALK5) (Fig. 6.1). After binding to its receptors, TGF-β induces phosphorylation of TβRI, which phosphorylates receptor-associated Smad2 and Smad3 (R-Smads). Phosphorylated Smad2 and

Smad3 interact with Smad4. The Smad2, -3, -4 complex accumulates within the nucleus, binds to DNA, and regulates transcription of target genes. In addition to this "canonical" Smad signaling pathway, TGF-β activates Smad-independent pathways (MAPK, RHOA, PI3K/AKT) (Mu et al. 2012).

For a comprehensive review of the signaling events and molecular mechanisms by which TGF-β orchestrated cell growth arrest and apoptosis, the reader is invited to read Chap. 5. Escape from the anti-proliferative effects of TGF-β signaling is an obligate step for almost all tumors of epithelial origin. For this, cancer cells have positively selected inactivating mutations affecting genes coding for key players involved in TGF-β signaling, such as the *TGF-β* receptor genes and *Smad* genes. These mutations render cancer cells insensitive to the growth-inhibiting action of TGF-β. Here we shall focus on those mutations which have provided the information most relevant to better understanding the tumor-suppressive role of TGF-β (mainly Smad4, TβRII, and Smad2 mutations) (Fig. 6.1). For a more complete list of mutations affecting TGF-β signaling in human tumors, the reader is invited to consult comprehensive reviews published by others (Kim et al. 2000; Levy and Hill 2006; Massague 2008).

Germline mutations in *Smad4* or *BMPRIA* (bone morphogenetic protein receptor, type IA) are found in over 50 % of cases of juvenile polyposis syndrome (JPS) (Aretz et al. 2007; Howe et al. 1998; van Hattem et al. 2008). JPS patients develop during childhood or adolescence multiple polyps in the gastrointestinal tract, associated with a high risk of colorectal cancer (50 % by the age of 60 years). Beyond these germline mutations concerning a minority of cancer patients, somatic mutations compromising the TGF-β signaling pathway are recurrently found in many sporadic tumors (Fig. 6.1). For instance, the locus 18q21.1 containing *Smad4* is deleted in over 50 % of human pancreatic cancers (this locus is also known as *DPC4, Deleted in Pancreatic Carcinoma 4*) (Hahn et al. 1996). Mutation of *Smad4* appears, indeed, as a preferential means of tumor escape from the tumor-suppressive functions of TGF-β signaling, since Smad3 is practically never mutated in human neoplasms and Smad2 mutations are rather rare. Why this is so remains unclear, but it has been hypothesized that Smad4, being involved in the signaling of all the TGF-β superfamily members, might additionally inactivate BMP signaling, which is also involved in tumor suppression. This hypothesis is supported by the presence of BMPRIA mutations in a subset of human JPS patients (Howe et al. 2001). Alternatively, Smad2 and Smad3 might be relatively spared by genetic alterations as compared to Smad4 because of an “R-Smad dependency” of many tumors. It is tempting to speculate that retention of Smad2 and Smad3 might confer a selective advantage to the tumor by driving invasive programs (epithelial-to-mesenchymal transition - EMT, immunosuppression, extracellular remodeling). For instance, Smad2 and Smad3 still accumulate in the nucleus in Smad4-deficient human pancreatic cancer cells (Subramanian et al. 2004) suggesting that R-associated Smad proteins have nuclear functions independently of Smad4. This hypothesis is comforted by the Smad4-independent association of Smad2 and Smad3 with inhibitor of nuclear factor κ-B kinase subunit alpha (IKKα) (Descargues et al. 2008) or transcriptional intermediary factor 1 γ (TIF1γ) (He et al. 2006).

TβRII is rarely altered in pancreatic cancers but is deleted in the majority of colorectal cancers associated with microsatellite instability (MSI) (Markowitz et al. 1995). Why is it that in MSI colon cancer mutations occur more frequently in TβRII than in any other gene involved in TGF-β signaling? MSI colon cancers are characterized by defective DNA mismatch repair (MMR), so the tumors accumulate mutations in microsatellite sequences. The TβRII gene contains "microsatellite-like" sequences rendering it particularly vulnerable to mutations in cases of MSI colorectal cancer. Interestingly, TβRII mutations resulting from MSI are positively selected, enabling the tumor to escape the growth-inhibiting effect of TGF-β (Biswas et al. 2008). This is a genuine example of the capacity of human neoplasms to escape the tumor-suppressive effect of TGF-β thanks to preexisting intrinsic tumor properties (microsatellite instability, in the case of MSI colorectal tumors).

Genome-scale exon sequencing has confirmed that TGF-β signaling mutations in the genes encoding the TβRII and Smad proteins are a hallmark of certain types of cancer, as illustrated by the sequencing of 24 pancreatic tumors (Jones et al. 2008) and 276 colorectal cancers (Cancer Genome Atlas Network 2012). In contrast, TGF-β mutations failed to emerge as a tumor signature from a study on 92 medulloblastomas (Pugh et al. 2012) and 100 breast tumors (Stephens et al. 2012). This raises the question: how do cancer cells having no recurrent genomic alteration that compromises TGF-β signaling escape TGF-β-mediated growth inhibition? One should consider, firstly, that some tumors derive from cells that are not sensitive to TGF-β growth inhibition in the first place. This is the case of sarcomas, which derive from mesenchymal cells intrinsically insensitive to the cytostatic action of TGF-β and which therefore do not require impairment of the TGF-β signaling pathway in order to develop. Secondly, tumors displaying a low rate of mutation in genes involved in TGF-β signaling may have escaped the tumor-suppressive effect of TGF-β by other means. For instance, repression of TGF-β receptor gene transcription by DNA methylation has been reported in a human gastric cancer (Kang et al. 1999) and human breast tumors (Hinshelwood et al. 2007). Also reported in human tumors is up-regulation of negative regulators of TGF-β signaling. For instance, SKI/SNON expression is increased in breast cancer (Zhang et al. 2003), colorectal cancer (Buess et al. 2004), melanoma (Medrano 2003; Poser et al. 2005; Reed et al. 2001), and esophageal squamous cell carcinoma (Fukuchi et al. 2004).

6.2 Mouse Models of TGF-β-Induced Tumor Suppression

Many mouse models have been developed to decipher the role of TGF-β in cancer. They rely on introducing into the murine genome the human mutations described in paragraph 1 with the ultimate goal to compromise in vivo the tumor-suppressive functions described in paragraph 2.

Homozygous TGF-β1^{null} mice develop autoimmune disorders, culminating in their death by 3 weeks of age (Kulkarni et al. 1993; Shull et al. 1992). Half of the TGF-β1^{null} embryos die in utero because of hematopoiesis and vasculogenesis

Table 6.1 Mouse models with whole-body homozygous inactivation of central regulators of the TGF-β signaling

Knockout gene	References	Phenotype
TGF-β1^{null}	Shull et al. (1992)	Multifocal inflammatory disease, death before 1 month
	Kulkarni et al. (1993)	Multifocal inflammatory disease, death before 1 month
	Dickson et al. (1995)	Multifocal inflammatory disease, death before 1 month, 50 % of death at E10.5 because of defective hematopoiesis and vasculogenesis
TGF-β2^{null}	Sanford et al. (1997)	Wide range of developmental defects causing perinatal death (cardiac, lung, craniofacial, limb, spinal column, eye, inner ear, and urogenital defects)
TGF-β3^{null}	Proetzel et al. (1995)	Failure of the palatal shelves to fuse leading to cleft palate
TβRI/ALK5^{null}	Larsson et al. (2001)	Death at E10.5 because of defective hematopoiesis and vasculogenesis
TβRII^{null}	Oshima et al. (1996)	Death at E10.5 because of defective hematopoiesis and vasculogenesis
TβRIII^{null}/Betaglycan	Stenvers et al. (2003)	Embryonic lethality caused by defects in heart and liver
Smad2^{null}	Nomura and Li (1998) Waldrip et al. (1998) Weinstein et al. (1998)	Embryonic death because gastrulation defect
Smad3^{null}	Zhu et al. (1998)	Colorectal cancers
Smad4^{null}	Yang et al. (1998)	Death at E8.5, gastrulation defect
	Sirard et al. (1998)	Death at E8.5, gastrulation defect
	Takaku et al. (1998)	Death at E8.5, gastrulation defect

defects in the yolk sac (Dickson et al. 1995). TGF-β2^{null} (Sanford et al. 1997) and TGF-β3^{null} (Proetzel et al. 1995) mice are viable, but display multiple developmental defects. Homozygous knockouts of TβRI/ALK5 (Larsson et al. 2001) or TβRII (Oshima et al. 1996) are embryonically lethal. Knockout TβRIII (Betaglycan) mice develop embryonic lethal proliferative defects in heart and apoptosis in liver (Stenvers et al. 2003). Smad3^{null} mice spontaneously develop colorectal cancers (Zhu et al. 1998). Inactivation either of the other two Smad proteins involved in TGF-β signaling, Smad2 (Nomura and Li 1998; Waldrip et al. 1998; Weinstein et al. 1998) and Smad4 (Sirard et al. 1998; Takaku et al. 1998; Yang et al. 1998), leads to embryonic death (Table 6.1).

Altogether, these mouse models provide a perfect illustration of the pleiotropic effects of TGF-β and of its crucial role in development and homeostasis, as all these whole-organism mutations lead to embryonic or perinatal lethality (with the exception of Smad3 inactivation, responsible for colorectal tumors). As the severity of the induced phenotypes limits the utility of these models in cancer studies, several strategies have been devised to get around this limitation. In this paragraph, we will describe in detail informative mouse models that have been obtained by targeting

the mammary gland, digestive tract, exocrine pancreas. Mouse models of skin cancer, which constitute appropriate systems for exploring the effects of external carcinogens such as chemicals and ultraviolet irradiation, will be described in Chap. 9. Skin models along with models targeting other organs [e.g., the liver (Tang et al. 1998), prostate (Kundu et al. 2000; Pu et al. 2009), or head and neck (Bian et al. 2009; Bornstein et al. 2009)] are listed in Table 6.2 but are not extensively described in the text.

Table 6.2 Mouse models demonstrating the tumor-suppressive role of TGF-β

Organ	Genetic alteration	Organ specificity	Oncogenic cooperation	References
Breast	TGF-β1$^{S223/225}$	Yes	Alone	Pierce et al. (1993)
	TGF-β1$^{S223/225}$	Yes	Alone	Jhappan et al. (1993)
	TGF-β1$^{S223/225}$	Yes	Alone	Kordon et al. (1995)
	TGF-β1$^{S223/225}$	Yes	TGF-α	Pierce et al. (1995)
	TGF-β1$^{S223/225}$	Yes	Alone	Buggiano et al. (2001)
	TGF-β1$^{S223/225}$	Yes	Alone	Boulanger and Smith (2001)
	TGF-β1$^{S223/225}$	Yes	Neu	Muraoka et al. (2003)
	TGF-β1$^{S223/225}$	Yes	Alone	Boulanger et al. (2005)
	TGF-β3-KO	Whole body	Alone	Nguyen and Pollard (2000)
	TβRI^{CA}	Yes	PyVmT	Muraoka-Cook et al. (2004)
	TβRI^{CA}	Yes	Neu	Muraoka-Cook et al. (2006)
	TβRII^{DN}/TβRI^{CA}	Yes	Neu	Siegel et al. (2003)
	TβRII^{DN}	Yes	DMBA	Bottinger et al. (1997a)
	TβRII^{DN}	Yes	Alone	Gorska et al. (1998)
	TβRII^{DN}	Yes	None and TGF-α	Gorska et al. (2003)
	TβRII-KO^{het}	Yes	PyVmT	Fang et al. (2011)
	TβRII-KO^{null}	Yes	PyVmT	Forrester et al. (2005)
	TβRII-KO^{null}	Yes	Alone	Cheng et al. (2005)
	TβRII-KO^{null}	Yes	Alone	Yang et al. (2008)
	TβRII-KO^{null}	Yes	PyVmT	Bierie et al. (2008)
	TβRII-KO^{null}	Yes	PyVmT	Bierie et al. (2009)
	None (treatment with TβRII blocking antibody)	N/A	PyVmT	Muraoka et al. (2002)
	Smad4-KO^{null}	Yes	Alone	Li et al. (2003)
	Smad3-KO^{null}	Whole body	Alone	Yang et al. (2002)
Intestine	TGF-β1^{null}	Whole body	Rag2^{null}	Engle et al. (2002)
	TGF-β1^{null}	Whole body	Rag2^{null}	Engle et al. (1999)
	TβRI-KO^{het}	Whole body	Apc^{Min}	Zeng et al. (2009)

(continued)

Table 6.2 (continued)

Organ	Genetic alteration	Organ specificity	Oncogenic cooperation	References
	TβRII-KO^{null}	Yes	Azoxymethane (AOM)^{null}	Biswas et al. (2004)
	TβRII-KO^{null}	Yes	Apc^{1638N}	Munoz et al. (2006)
	TβRII-KO^{null}	Yes	KRAS^{G12D} and Apc^{1638N}	Trobridge et al. (2009)
	Smad2-KO^{het}	Whole body	ApcΔ^{716}	Takaku et al. (2002)
	Smad2-KO^{het}	Whole body	Apc^{580D}	Hamamoto et al. (2002)
	Smad3-KO^{null}	Whole body	Alone	Zhu et al. (1998)
	Smad3-KO^{null}	Whole body	Helicobacter spp.	Maggio-Price et al. (2006)
	Smad3-KO^{null}	Whole body	ApcMin	Sodir et al. (2006)
	Smad4-KO^{het}	Whole body	ApcΔ^{716}	Takaku et al. (1998)
	Smad4-KO^{het}	Whole body	Alone	Takaku et al. (1999)
	Smad4-KO^{het}	Whole body	Alone	Xu et al. (2000)
	Smad4-KO^{het}	Whole body	Alone	Hohenstein et al. (2003)
	Smad4-KO^{hetE6D}	Whole body	Apc^{1638N}	Alberici et al. (2006)
	Smad4-KO^{het}	Whole body	Elf^{het}	Redman et al. (2005)
	Smad4-KO^{het}	Whole body	Elf^{het}	Tang et al. (2005)
	Smad4-KO^{het}	Whole body	Elf^{het}	Katuri et al. (2006)
	TβRII^{fspKO}	Mesenchymal compartment	Alone	Bhowmick et al. (2004)
	Smad4-KO^{het}	Whole body	ApcΔ^{716}	Kitamura et al. (2007)
	Smad4-KO^{het}	Yes	Apc^{1638N}	Freeman et al. (2012)
	Smad4-KO^{het}	Yes	Alone	Kim et al. (2006)
Skin	TGF-β1$^{S223/225}$	Yes	Alone	Sellheyer et al. (1993)
	TGF-β1$^{S223/225}$	Yes	TPA	Cui et al. (1995)
	TGF-β1$^{S223/225}$	Yes	TPA	Fowlis et al. (1996)
	TGF-β1$^{S223/225}$	Yes	TPA	Cui et al. (1996)
	TGF-β1$^{S223/225}$	Yes	DMBA	Weeks et al. (2001)
	TGF-β1$^{S223/225}$	Yes	Alone	Liu et al. (2001)
	TGF-β1$^{S223/225}$	Yes	Alone	Wang et al. (1999)
	TβRII^{DN}	Yes	Alone	Wang et al. (1997)
	TβRII^{DN}	Yes	Alone	Amendt et al. (1998)
	TβRII^{DN}	Yes	TPA	Go et al. (1999)
	TβRII^{DN}	Yes	TPA,DMBA	Go et al. (2000)
	TβRII^{DN}	Yes	Alone	Amendt et al. (2002)
	TβRII^{DN}+ TβRII-KO^{null}	Yes	DMBA/TPA	Han et al. (2005)
	TβRII-KO^{null}	Yes	Ha-Ras^{V12}	Guasch et al. (2007)
	Smad3-KO^{null}	Whole body	Alone	Ashcroft et al. (1999)
	Smad4-KO^{het}	Whole body	Elf^{het}	Redman et al. (2005)
	Smad4-KO^{null}	Yes	Alone	Li et al. (2003)

(continued)

Table 6.2 (continued)

Organ	Genetic alteration	Organ specificity	Oncogenic cooperation	References
	Smad4-KO^{null}	Yes	PTEN $^{-/-}$	Yang et al. (2005)
	Smad4-KO^{null}	Yes	Alone	Qiao et al. (2006)
Pancreas	TβRII^{DN}	Whole body	Alone	Bottinger et al. (1997b)
	TβRII-KO^{null}	Yes	K-Ras^{G12D}	Ijichi et al. (2006)
	TβRII-KO^{null}	Yes	K-Ras^{G12D}	Ijichi et al. (2011)
	Smad4-KO^{null}	Yes	K-Ras^{G12D}	Bardeesy et al. (2006)
	Smad4-KO^{null}	Yes	K-Ras^{G12D}	Kojima et al. (2007)
	Smad4-KO^{null}	Yes	K-Ras^{G12D}	Izeradjene et al. (2007)
	Smad7^{Tg}	Yes	Alone	Kuang et al. (2006)
	TIF1γ-KO^{null}	Yes	K-Ras^{G12D}	Vincent et al. (2009)
	TIF1γ-KO^{null}	Yes	K-Ras^{G12D}	Vincent et al. (2012)
	NupR1-KO^{null}	Whole body	K-Ras^{G12D}	Cano et al. (2012)
Head and Neck	TβRI-KO^{null}	Yes	DMBA	Bian et al. (2009)
	Smad4-KO^{null}	Yes	Alone	Bornstein et al. (2009)
Prostate	TβRII^{DN}	Yes	SV40-Large T	Pu et al. (2009)
	TβRII^{fspKO}	Mesenchymal compartment	none	Bhowmick et al. (2004)
	TβRII^{DN}	Yes	none	Kundu et al. (2000)
Liver	TGF-β1^{het}	Whole body	Diethylnitro-samine (DEN)	Tang et al. (1998)
	Smad3^{Tg}	Yes	Diethylnitro-samine (DEN)	Yang et al. (2006)

6.2.1 The Mammary Gland

In mice, mammary gland development (Sternlicht 2006) begins shortly after mid-gestation (E10.5). It is characterized by the presence at birth of a rudimentary system of branching ducts opening into the nipple. From birth to puberty, there is but little development of the branching duct network. At puberty (~5 weeks of age), a massive wave of branching occurs, so that the ducts fill the entire mammary fat pad of the young adult. During the estrus cycles, the mammary gland undergoes successive cycles of proliferation and regression. Pregnancy is associated with massive cell proliferation in the mammary gland, along with lobular-alveolar differentiation to produce milk. Lactation in the mother's mammary gland starts when the pups are born. After weaning, lactation stops and the secreting lobular-alveolar network of the mammary gland involutes, an event resulting from massive apoptosis.

In the TGF-β1$^{S223/225}$ mutant, cysteine residues at positions 223 and 225 are replaced with serine residues so that TGF-β1 is secreted in an active form (this mutant is unable to bind the latency-associated protein, LAP). Transgenic mice expressing TGF-β1$^{S223/225}$ have been generated, with expression of the TGF-β1$^{S223/225}$ transgene under the control of either the promoter of the mouse mammary tumor

virus (MMTV) gene (associated with early differentiation of the mammary gland) or the promoter of the whey acidic protein (WAP) gene (associated with late pregnancy, lactation, and early involution). In MMTV-TGF-β1$^{S223/225}$ mice (Pierce et al. 1993), duct development is severely compromised, this leading to mammary gland hypoplasia. WAP-TGF-β1$^{S223/225}$ mice develop normal ducts but are not capable to support lactation as they present a severe deficiency in their ability to form secretory lobules during pregnancy (Jhappan et al. 1993) as a consequence of the early senescence of the regenerative capacity of the mammary ductal epithelium (Kordon et al. 1995). TGF-β3 expression from the β-lactoglobulin promoter in the lactating epithelium of mice is likewise reported to induce involution of the mouse mammary gland (Nguyen and Pollard 2000). Models have also been developed to inhibit TGF-β signaling in the mammary gland. As expected, these models give rise to phenotypes opposite to those just described. Thus, virgin MMTV-TβRII^{DN} (dominant negative mutant form of TβRII) (Bottinger et al. 1997b; Gorska et al. 1998) and MMTV-Cre/TβRII-KO$^{L/L}$ (a conditional TβRII-knockout allele) (Forrester et al. 2005) mice display hyperplasia and differentiation of the mammary glands. The MMTV-TβRII^{DN} model shows delayed involution of the mammary gland after forced weaning of pups during lactation (Gorska et al. 2003).

Smad3^{null} mice, which develop colorectal tumors (Zhu et al. 1998), also show under-developed mammary glands and involution defects (Yang et al. 2002). The mammary glands of WAP-Cre/TβRII-KO$^{L/L}$ mice likewise fail to undergo complete involution of the lobular-alveolar secreting structures (Bierie et al. 2009). Defective mammary gland involution is due to compromised apoptosis, a crucial biological process controlled by TGF-β. Unexpectedly, MMTV-Cre/Smad4$^{L/L}$ and WAP-Cre/Smad4$^{L/L}$ mice display no involution defects or obvious developmental defects (Li et al. 2003), but they do show spontaneous mammary tumor formation (Li et al. 2003). The mammary glands of MMTV-Cre/Smad4-KO$^{L/L}$ mice show increased proliferation, alveolar hyperplasia, and trans-differentiation into squamous epithelial cells, and they finally develop squamous cell carcinoma (SCC). To date, this is the only model of spontaneous mammary tumor formation in genetically engineered mice with compromised TGF-β signaling. The development of mammary tumors when Smad4 is knocked down may be due to more efficient inactivation of TGF-β signaling than in TβRII^{DN} mice (Bottinger et al. 1997b; Gorska et al. 1998). Alternatively, it might be due to inactivation of a non-TGF-β pathway such as the BMP pathway, which should occur upon Smad4 knockdown but not in the TβRII-KO model (Forrester et al. 2005).

Altogether, these observations demonstrate that TGF-β exerts anti-proliferative effects constraining mammary duct development in virgin mice and enabling involution of the mammary gland at the end of lactation. They also functionally validate the pivotal role of Smad4 and TβRII in limiting cell proliferation and tumor formation, in line with the high prevalence of genetic alterations affecting both genes in human neoplasms.

Because disruption of TGF-β signaling is not sufficient to induce mammary tumors in most of the models described above (only Smad4 inactivation is sufficient to induce mammary tumors), mutations affecting TGF-β signaling have been

combined with various oncogenic factors. This has yielded robust models of predisposition to mammary tumors. These approaches include:

1. Exploring the effects of chemical carcinogens in mice with attenuated TGF-β signaling. It appears that MMTV-Cre/TβRII^{DN} mice are more sensitive to 7,12-dimethylbenz(α)anthracene (DMBA)-induced mammary tumorigenesis than control littermates (Bottinger et al. 1997b). This sensitization indirectly confirms the tumor-suppressive function of TGF-β.
2. Studying the role of TGF-β in relation to mammary tumorigenesis in multiparous females. MMTV-Cre/TβRII^{DN} mice having experienced two pregnancies with partial involution of the secreting lobular-alveolar structures have an increased risk of mammary tumor formation (Gorska et al. 2003). This observation suggests that TGF-β is a potent inhibitor of the transformation of hyperplastic tissues, known to be more likely to evolve towards neoplastic lesions. This observation identifies TGF-β-induced apoptosis, responsible for mammary gland involution after lactation, as a crucial tumor-suppressive mechanism.
3. Assessing susceptibility to the development of mammary tumors after MMTV infection. Milk-transmitted MMTV infection has been extensively used to induce mammary tumors [for reviews, see (Callahan and Smith 2000; Ross 2010)]. This line of research has yielded contradictory results: one group found WAP-TGF-β1$^{S223/225}$ mice to show no change in susceptibility to MMTV infection or subsequent tumor development, despite a severe decrease in lobular alveolar development (Buggiano et al. 2001); another group found WAP-TGF-β1$^{S223/225}$ mice to display a decreased risk of mammary cancer after MMTV infection (Boulanger and Smith 2001), thanks to early senescence in the mammary stem cell population (Boulanger et al. 2005). Although the reason for this discrepancy is unclear, it is important to note that TGF-β may prevent mammary tumors by inducing the senescence of stem cells, in addition to its pro-apoptotic effect on differentiated cells.
4. Exploiting oncogene-induced mouse models of mammary cancer. Three oncogenes are classically used in transgenic mice: transforming growth factor alpha (TGF-α), Her-2/neu (erbB-2), and polyoma virus middle T antigen (PyVmT). MMTV-TGF-β1$^{S223/225}$/MMTV-TGF-α mice develop significantly fewer mammary tumors than MMTV-TGF-α mice (Pierce et al. 1995). Conversely, MMTV-Cre/TβRII-KO$^{L/L}$/MMTV-PyVmT mice develop tumors more quickly than MMTV-PyVmT mice (Forrester et al. 2005), and MMTV-TβRII^{DN}/MMTV-TGF-α develop mammary tumors at a higher frequency than MMTV-TGF-α mice (Gorska et al. 2003). WAP-Cre/TβRII-KO$^{L/L}$/MMTV-PyVmT mice also present an accelerated mammary tumorigenesis (Bierie et al. 2008). Finally, MMTV-TβRI^{CA}/MMTV-Neu mice develop primary tumors later (Siegel et al. 2003) and MMTV-TβRIIDN/MMTV-Neu mice develop them earlier than MMTV-Neu mice (Siegel et al. 2003). Together, these results indicate that TGF-β efficiently opposes TGF-α-, PyVmT-, and Her2/Neu- oncogene-driven transformation of the epithelial mammary tissue, illustrating once again its tumor-suppressor role.

5. Disrupting TGF-β signaling in the stromal compartment instead of the epithelial compartment. This approach relies on a mouse model in which TGF-β signaling is specifically abolished in the mesenchymal compartment and involves associating a Cre-recombinase controlled by the FSP1 (fibroblast specific protein-1; S100A4) promoter with a conditional TβRII-knockout allele in FSP1-Cre/TβRII$^{L/L}$ compound transgenic mice. These mice develop pre-neoplastic lesions resulting in prostate and stomach carcinomas and display severely compromised development of the mammary gland (Bhowmick et al. 2004). Furthermore, it appears that TβRII-deficient fibroblasts can facilitate tumor growth when grafted with PyVmT or 4T1 mammary carcinoma cells (Cheng et al. 2005). This finding has been further validated in MMTV-PyVmT/FSP1-Cre/TβRII$^{L/+}$ mice developing autochthonous mammary tumors (Fang et al. 2011). Finally, xenografts onto the chicken embryo chorioallantoic membrane revealed that the absence of TβRII in fibroblasts could promote invasiveness of murine mammary carcinoma cells (Matise et al. 2012). Altogether, these studies clearly demonstrate that the tumor-suppressive function of TGF-β relies also on microenvironmental effects.

Importantly, although this point lies outside the scope of this chapter, the mammary gland is particularly suitable for investigating the dual role of TGF-β during carcinogenesis, since many studies based on mouse models of mammary tumors indicate that once transformation has occurred and a tumor has become established, TGF-β actually facilitates tumor progression and subsequent metastatic dissemination, in contrast to its tumor-suppressive effect on the normal and hyperplastic mammary gland (Bottinger et al. 1997a; Forrester et al. 2005; Gorska et al. 2003; Muraoka-Cook et al. 2004; Muraoka-Cook et al. 2006; Muraoka et al. 2002; Muraoka et al. 2003). Tumor-promoter functions of TGF-β are developed in Chap. 7.

6.2.2 The Digestive Tract

The digestive tract is anatomically divided into different parts: the mouth, esophagus, stomach, small intestine, colon, and rectum. The intestinal lumen is lined with a specialized epithelium organized into crypts and villi (van der Flier and Clevers 2009). Stem and proliferating cells are located in the crypts, whereas differentiated cells (enterocytes, entero-endocrine cells, and goblet cells) are located in the villi. Paneth cells constitute the only differentiated cell population settling in the crypts (they do not migrate "upward"). Colorectal adenocarcinoma is the most frequent tumor of the digestive tract. In 1990, Fearon and Vogelstein proposed a model according to which this cancer arises through an ordered sequence of mutations called the adenomacarcinoma sequence (Fearon and Vogelstein 1990). As mentioned earlier in this chapter, the spontaneous development of colorectal tumors in Smad3^{null} mice constitutes strong evidence of the tumor-suppressive role of TGF-β

(Zhu et al. 1998). To explore more precisely the role of TGF-β signaling in cancers of the digestive tract, different cancer predisposition models have been developed:

1. Confirmation of the tumor-suppressive effect of TGF-β signaling on digestive tract tumorigenesis was first obtained in heterozygous Smad4-KO^{het} mice. These mice develop polyps in the proximal gastrointestinal tract, confirming the central role of Smad4 in tumor suppression (Takaku et al. 1999; Xu et al. 2000). Smad4-KOE6sad (sad, serrated adenomas) is a null mutant allele caused by a spontaneous deletion in a splice acceptor site, sufficient to induce serrated adenomas and mixed polyposis (Alberici et al. 2006). Interestingly, knocking down Smad4 in T-lymphocytes (CD4-Cre/Smad4-KO$^{L/L}$) results in epithelial tumors of the oral cavity, stomach, duodenum, rectum, and colon (Kim et al. 2006). This recent observation constitutes new evidence that TGF-β signaling in the microenvironment plays an active role in constraining epithelial tumorigenesis.
2. The best-represented category of digestive tract cancer predisposition models relies on associating TGF-β signaling mutations with mutations that inactivate the adenomatous polyposis coli (APC) gene, which encode an inhibitor of the WNT/β-catenin signaling pathway (van der Flier and Clevers 2009). This pathway is particularly important in the digestive tract, as it maintains the crypt as a "proliferative, undifferentiated, and multipotent" compartment. In contrast, the WNT/β-catenin is not active in the villi, which constitute the "differentiated" compartment. In the absence of WNT ligands, β-catenin binds to a complex containing GSK3β (glycogen synthase kinase-3β), CK1 (casein kinase 1), axin (conductin), and APC. This complex phosphorylates β-catenin, targeting it for ubiquitination and degradation. In the presence of WNT ligands, the receptors Frizzled and LRP (low-density lipoprotein receptor-related protein) are activated, leading to inhibition of the "destruction complex" and stabilization of β-catenin, which can accumulate inside the nucleus and eventually activate transcription of growth-promoting genes. The canonical WNT signal transduction pathway is frequently impaired in human cancers, especially those arising in the digestive tract. As the most common alterations of the WNT/β-catenin pathway in cancer are APC-inactivating and β-catenin-activating mutations, several mouse models have been developed with APC mutations. The different mutants develop small intestinal polyps that are histologically indistinguishable, but the average number of polyps is different according to the mutation: 3 in APC^{1638N} mutants, 30 in APC^{Min} (Min, multiple intestinal neoplasia) mutants, and 300 in APCΔ^{716} mutants. These mouse models are somewhat evocative of human familial adenomatous polyposis (FAP, caused by deletions on chromosome 5q encompassing the APC gene), but in contrast to mice with APC mutations, human FAP patients, who present an increased risk of colorectal cancer, develop polyps mainly in the colon and rectum (not in the small intestine).

   Because TGF-β is impaired in colorectal cancers as a result of mutations affecting the genes encoding the TGF-β receptors and Smad proteins, these

mutations have been combined with APC mutations in transgenic mouse models. Whereas mice homozygous for a Smad3-inactivating mutation spontaneously develop colorectal cancers between 18 and 24 weeks of age (Zhu et al. 1998), APC^{min}/$Smad3^{null}$ mice develop tumors much earlier and are moribund by the age of 8 weeks (Sodir et al. 2006). $APC\Delta^{716}$/Smad4-$KO\Delta^{Ex1/+}$ mice develop colorectal cancers (Takaku et al. 1998) and permitted the identification of a new type of immature myeloid cell (iMC), expressing matrix metalloproteinases (MMP9 and MMP2) and the CC-chemokine receptor 1 (CCR1), present at the invasion front toward CCL9 (the CCR1 ligand), to facilitate tumor invasion (Kitamura et al. 2007). Inactivation of CCR1 in $APC\Delta^{716}$/Smad4-$KO\Delta^{Ex1/+}$/CCR1-KO^{null} compound mice inhibits the accumulation of iMCs preventing the tumor invasion. Whereas Smad4-$KO^{E6sad/+}$ (Hohenstein et al. 2003) develop serrated adenomas and mixed polyposis, APC^{1638N}/Smad4-$KO^{E6sad/+}$ mice have been shown to develop aggressive colorectal cancers (Alberici et al. 2006). As observed in human tumors, the onset of colorectal tumors in mouse models is systematically associated with Smad4 loss of heterozygosity (LOH), indicating that total loss of Smad4 function is required for transformation, rather than haploid insufficiency. Heterozygous inactivation of Smad2 in a mutated APC background was shown in one study to accelerate tumor progression (Hamamoto et al. 2002) and in another study to have no effect (Takaku et al. 2002). The cause of this discrepancy remains unclear but may be due to the use of different mutant APC alleles. Heterozygous inactivation of TβRI in association with the APC^{Min} mutation is associated with a twofold increase in the number of intestinal tumors and with colorectal cancer onset without loss of heterozygosity at the TβRI locus (Zeng et al. 2009). Thus, in contrast to what is observed upon Smad4 inactivation, haploid insufficiency of TβRI is sufficient to facilitate tumorigenesis. Finally, Villin-Cre-dependent activation of the conditional TβRII-$KO^{L/L}$ conditional allele is associated with colorectal cancer development in an APC^{1638N} background (Munoz et al. 2006). The molecular interaction between the TGF-β signaling and the WNT/β-catenin signaling has been recently described by performing microarray performed with biological material prepared from adenomas from $APC\Delta^{1638/+}$/K19-Cre-ERT2/$Smad4^{L/L}$, which revealed increased levels of β-catenin mRNA and WNT signaling (Freeman et al. 2012).

Altogether, these models show that TGF-β-pathway-inactivating mutations strongly potentiate the capacity of APC mutants to induce colorectal cancers. These mouse models clearly demonstrate that progression of colorectal cancers involves inactivation of TGF-β signaling illustrating its tumor suppressor effect. Notably, by combining APC mutations with TGF-β signaling mutations, it is possible to better mimic human FAP, as the lesions appear in the distal intestine.

3. A third category of digestive tract cancer predisposition models relies on the association of TGF-β signaling mutations with mutations affecting genes other than APC. The ELF protein (embryonic liver fodrin), identified in foregut progenitors, belongs to the β-spectrin family involved in cell polarity (Machnicka

et al. 2012). Double heterozygous Smad4-KO^{het}/ELF-KO^{het} mutants develop colorectal cancers and also gastric and liver tumors (Katuri et al. 2006; Redman et al. 2005; Tang et al. 2005). Activating KRAS mutations are classically associated with the transition from the adenoma to the carcinoma state. LSL-KRAS^{G12D} (LSL, Lox-Stop-Lox) is a conditional (Cre-inducible) allele encoding a constitutively active KRAS mutant (KRAS^{G12D}). Villin-Cre/LSL-KRAS^{G12D}/TβRII-KO$^{L/L}$ mice develop small intestinal and colorectal carcinomas by the age of 20 weeks (Trobridge et al. 2009).

4. A fourth strategy for producing digestive tract cancer predisposition models involves mice with different genetic backgrounds to avoid the lethal inflammatory phenotype observed in mice with compromised TGF-β signaling due to TGF-β or Smad inactivation. To avoid the generalized inflammatory disorders that cause early death of TGF-β1^{null} mice at ~3 weeks of age (Kulkarni et al. 1993; Shull et al. 1992), homozygous inactivation of TGF-β1 was introduced into immunodeficient RAG2^{null} mice, lacking both B and T cells. TGF-β1^{null}/RAG2^{null} double-mutant mice do not develop inflammatory disease, live to adulthood, and develop colorectal cancers by the age of 5 months (Engle et al. 1999). Interestingly, if these mice are made germ-free, they no longer develop colorectal cancer; this indicates that microbe-induced inflammation is crucial to the development of colon cancer (Engle et al. 2002). In the 129/Sv background, all Smad3^{null} mice develop tumors by 6 months of age, whereas in the hybrid 129/Sv;C57BL/6 background, only 30 % of these mice develop tumors (Zhu et al. 1998). Once again, the crucial role of the microenvironment is illustrated.
5. The last approach to developing digestive tract cancer predisposition models involves treating transgenic mice with external agents facilitating colorectal transformation. Indeed, although embedded in the body, the lumen of the digestive tract is in fact in direct contact with the external environment, rendering it more vulnerable to external aggressions. Fatty-acid-binding (FABP)-promoter-mediated Cre recombination makes it possible to target floxed alleles to the colonic crypts (Saam and Gordon 1999). FABP-Cre/TβRII-KO$^{L/L}$ mice displaying targeted inactivation of TβRII in the distal intestine do not develop tumors of the digestive tract but are more sensitive than their "control" littermates to colorectal carcinogenesis induced by azoxymethane (a chemical carcinogen) (Biswas et al. 2004). In a helicobacter-free environment, furthermore, Smad3^{null} mice do not develop colon cancer by the age of 9 months, and infection of these mice induces colon cancer in >50 % of the animals (Maggio-Price et al. 2006). Finally, it has been shown that TGF-β-KO^{het} mice developed more liver and lung tumors after treatment with diethylnitrosamine (DEN) (Tang et al. 1998), whereas transgenic mice overexpressing Smad3 are partially protected against DEN-induced liver carcinogenesis as a result of increased apoptosis (Yang et al. 2006). This again illustrates the crucial role of TGF-β-induced apoptosis in tumor suppression.

6.2.3 The Exocrine Pancreas

The pancreas comprises separate functional units that regulate two major physiological processes: digestion and glucose metabolism [for review, see (Gittes 2009)]. The exocrine pancreas consists of acinar and duct cells. The acinar cells produce digestive enzymes and constitute the bulk of the pancreatic tissue. They are organized into grape-like clusters at the smallest termini of the branching duct system. The ducts, which add mucous and bicarbonates to the enzyme mixture, form a network of increasing size, culminating in main and accessory pancreatic ducts that discharge their content into the duodenum. The endocrine pancreas, consisting of specialized cell types organized into compact islets embedded within the acinar tissue, secretes hormones (glucagon, insulin, pancreatic polypeptide, and somatostatin) into the bloodstream.

Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive neoplasm, always lethal [for review, see (Kern et al. 2011)]. It is the fifth most common cause of death from cancer in the western world. At the time of diagnosis, the median age of PDAC-bearing patients is 65–70 years. PDAC affects the exocrine pancreas and accounts for ~80 % of pancreatic cancers. It arises from precursor lesions with differentiated ductal features. These precursor lesions are divided into three groups: pancreatic intraepithelial neoplasms (PanIN), intraductal papillary mucinous neoplasms (IPMN), and mucinous cystic neoplasms (MCN). Certain genetic alterations are recurrently found in sporadic and familial human PDAC, such as KRAS-activating mutations in over 90 % of cases and Ink4A/Arf- and TP53-inactivating mutations, each in about 50 % of cases [for review, see (Hansel et al. 2003) and (Landi 2009)]. The *SMAD4* gene is located at chromosomal locus 18q21, which is frequently lost in pancreatic cancer, especially in higher-grade lesions (Fukushige et al. 1998; Hahn et al. 1996; Schleger et al. 2000). Introduction of these mutations into mice has made it possible to create more than 20 models, recapitulating the whole spectrum of lesions observed in humans (according to the standard classification described in (Hruban et al. 2001), (Hruban et al. 2006) and http://pathology.jhu.edu/pancreas_panin/). Most of the models developed so far rely on targeted conditional expression in the Lox-Stop-Lox $KRAS^{G12D}$ (LSL-$KRAS^{G12D}$) knock-in mouse strain (Jackson et al. 2001), which bears a Cre-inducible allele targeting the endogenous KRAS locus with the most common alteration found in pancreatic cancers. Pancreas-specific activation of the LSL-$KRAS^{G12D}$ allele with the Pdx1-Cre (Gu et al. 2002) or Ptf1a/p48-Cre (Kawaguchi et al. 2002) allele induces the development of age-dependent precursor lesions (PanINs lesions in 100 % of animals) and PDAC after 1 year (in about 10 % of mice) (Hingorani et al. 2003). Combining the LSL-$KRAS^{G12D}$ allele with inactivation of the tumor suppressor Ink4A/Arf leads to very aggressive PDAC at a few weeks of age (median survival: approximately 2 months). This is the most robust and aggressive mouse model of PDAC developed to date (Aguirre 2003). This work and the unique opportunity offered by the Cre-lox system have prompted researchers to combine the $KRAS^{G12D}$ allele with many tumor suppressors inactivation and with oncogenic activations observed in humans (Bartholin 2012).

TGF-β signaling mutations combined with KRAS-activating mutations have emerged as very potent inducers of pancreatic cancer in mouse models, illustrating the tumor-suppressive role of TGF-β in this organ. These models provide a genuine opportunity to explore the complex role of TGF-β during carcinogenesis. Since Smad4 deletion is one of the most common genetic alterations in human PDAC, several mouse models have been developed where TGF-β signaling is specifically inactivated in the pancreas. Combined with KRAS activation, homozygous deletion of Smad4 induces cystic lesions, either IPMNs or MCNs (Bardeesy et al. 2006; Izeradjene et al. 2007; Kojima et al. 2007). Smad7 is a repressor of TGF-β signaling. Transgenic mice expressing Smad7 in the pancreas also develop PanINs (Kuang et al. 2006). Combined with TβRII inactivation targeted to the pancreas using the Ptf1a-Cre allele, $KRAS^{G12D}$ induces PDAC with a higher penetrance than observed with Smad4 (Ijichi et al. 2006), indicating that the tumor-suppressive function of TGF-β probably involves Smad4-independent mechanisms. Interestingly, PDAC cells in Ptf1a-Cre/LSL-$Kras^{G12D}$/$T\beta RII^{L/L}$ secrete high levels of several CXC chemokines, which in turn induce in the pancreatic stromal fibroblasts the expression of connective tissue growth factor (CTGF) , a profibrotic and tumor-promoting factor (Ijichi et al. 2011). This observation represents a novel evidence of the complex dialogue existing between the epithelial and the mesenchymal compartment during tumor progression.

Enlarged cells with vacuoles containing other cells have been observed by pathologists in human tumors for over 100 years. These “cells-in-cells” have notably been described in pancreatic cancer (Silverman et al. 1988; Tracey et al. 1984). At first, this cell cannibalism was hypothesized to be a pro-tumor mechanism, killing immune cells or feeding cancer cells upon starvation. More recently, a tumor-suppressive role for “cell-in-cells” process was proposed after the observation that cultured breast cancer cells could invade each other in vitro leading to a cell-death process that was called entosis or homotypic cell cannibalism (HoCC) (Overholtzer et al. 2007). Decreased HoCC has recently been reported in patients with metastasized PDAC (Cano et al. 2012), suggesting that HoCC plays a tumor-suppressive role in human pancreatic cancer. The HoCC decrease in the most aggressive PDACs is due to Nupr1. Nupr1is a stress protein activated at the transcriptional level by TGF-β (Pommier et al. 2012), overexpressed in late stages of PDAC and its metastases (Ito et al. 2005; Su et al. 2001a; b), involved in resistance to gemcitabine (the reference chemotherapy against PDAC) (Giroux 2006), and associated with a poor prognosis in PDAC patients (Hamidi et al. 2012). Nupr1 inhibits HoCC and enhances aggressiveness in PDAC by activating the EMT in response to TGF-β (Cano et al. 2012). Inactivation of Nupr1 in the $KRAS^{G12D}$/$Ink4A/Arf^{null}$ model (Pdx1-Cre/LSL-$KRAS^{G12D}$/Ink4A/Arf-$KO^{L/L}$/$Nupr1^{null}$ mice) results in a less aggressive phenotype characterized by enhancement of cell cannibalism. This validates the view that Nupr1 antagonizes cell cannibalism in vivo within the primary tumor. In Nupr1-depleted cells, unexpectedly, TGF-β stimulates HoCC (Cano et al. 2012). This suggests that HoCC is a tumor-suppressive program activated by TGF-β. The following model can be proposed: in the presence of Nupr1, TGF-β induces EMT and behaves as a tumor promoter; in the absence of Nupr1, TGF-β induces HoCC and behaves as a tumor suppressor.

Transcriptional intermediary factor 1γ (TIF1γ; alias, TRIM33/RFG7/PTC7/ectodermin) belongs to an evolutionarily conserved family of nuclear factors that have been implicated in stem cell pluripotency, embryonic development and tumor suppression (Hatakeyama 2011). Chromosomal breakpoints chromosome on 1p13.1 containing *TIF1γ* gene have been reported in acute megakaryocytic leukemias (Ng et al. 1999), osteochondromas (Sawyer et al. 2002), bronchial large cell carcinomas (Johansson et al. 1994), and childhood papillary thyroid carcinomas (Sawyer et al. 2002). Moreover, abrogation of the closely related *TIF1α* gene in mice caused hepatocellular carcinoma (Khetchoumian et al. 2007). These observations reinforce the idea according to which TIF1γ loss of function could play an active protective role during tumorigenesis. TIF1γ contributes to TGF-β signaling, although its precise functional role is not clear. Some data point toward TIF1γ as a negative regulator of the pathway through its capacity to mono-ubiquinate Smad4 and limit Smad4 nuclear accumulation (Dupont et al. 2005; 2009; Levy et al. 2007). In contrast, other studies have suggested that TIF1γ played an important positive role in transducing TGF-β signaling through its interaction with Smad2 and Smad3 (Bai et al. 2010; Doisne et al. 2009; He et al. 2006). For the reasons above mentioned, e.g., the involvement of TIF1γ in TGF-β signaling through its interaction with the Smad proteins, the location of TIF1γ at a locus that is altered in human tumors and the development of liver tumors in TIF1α knockout mice, a possible role of TIF1γ in pancreatic cancer was assessed. First, TIF1γ expression was reported to be markedly down-regulated in human pancreatic tumors (Vincent et al. 2009). Next, Pdx1-driven TIF1γ inactivation was shown to cooperate with the $KRAS^{G12D}$ oncogene in the mouse pancreas to induce exocrine pancreatic tumors (Vincent et al. 2009; Vincent et al. 2012). The relationship between *Tif1γ* and *Smad4* genes in pancreatic tumors was explored in double-null homozygous mutant mice in $KRAS^{G12D}$ background (Vincent et al. 2012) and revealed a pronounced genetic interaction between Smad4 and TIF1γ inactivation, the Pdx1-Cre; $KRAS^{G12D}$; Smad4-$KO^{L/L}$; TIF1γ-$KO^{L/L}$ (KSSTT) mice exhibiting accelerated tumor initiation (Vincent et al. 2012). The mechanism by which TIF1γ exerts its tumor-suppressive effect remains elusive. The increased tumor initiation observed when TIF1γ and Smad4 are simultaneously inactivated (compared to single gene inactivation) indicates that both genes may independently function as tumor suppressors of KRAS-driven pancreatic tumor. However, whether TIF1γ constrains transformation by modulating TGF-β functions, through mechanisms that are independent of Smad4, remains an open question and constitute a conceivable hypothesis consistent with the phenotype observed in Pdx1-Cre; LSL-$KRAS^{G12D}$; TRβII-$KO^{L/L}$ mice (Ijichi et al. 2006). Indeed, these mice, in which canonical and non-canonical TGF-β pathways are abrogated, present a phenotype resembling the one observed in KSSTT mice. Finally, it cannot be excluded that TIF1γ prevents transformation through mechanisms totally unrelated to TGF-β signaling, resulting from the modulation of general programs affecting broader genomic functions. For instance, TIF1γ and related family members were involved in chromatin remodeling, transcription elongation, and DNA repair (Agricola et al. 2011; Kepkay et al. 2011; Tsai et al. 2010). As briefly evocated in this chapter, after the malignant

transformation has occurred by bypassing its anti-proliferative and pro-apoptotic effects, TGF-β acquires oncogenic properties and eventually facilitates tumor progression. In some cases, TGF-β-mediated Smad4 activation may facilitate local invasion and metastatic dissemination by inducing EMT. In malignant tumors that retained a functional Smad4, it is possible that TIF1γ may behave as a tumor suppressor, through its capacity to mono-ubiquitinate Smad4 and limit its nuclear accumulation, thus limiting the aggressiveness of Smad4-positive cancer cells by compromising their capacity to undergo EMT. This hypothesis is supported both by in vitro and in vivo evidence. Indeed, it has been shown that TIF1γ could inhibit TGF-β-induced EMT of human mammary epithelial cells in culture (Hesling et al. 2011). In vivo, even if the double-null mice for TIF1γ and Smad4 develop pancreatic tumors with a shorter latency and a higher penetrance, these tumors present a well-differentiated phenotype contrasting with the poorly differentiated phenotype observed in TIF1γ null mice (Vincent et al. 2012). This phenotype may reflect the capacity of TIF1γ to inhibit Smad4-mediated EMT induced by TGF-β in later stages of tumorigenesis. TIF1γ inactivation cooperates with activated KRAS to promote the onset of poorly differentiated tumors in a Smad4-independent way. However, in vitro and in vivo evidence also supports that the absence of Smad4 could redirect TIF1γ-negative tumors toward a well-differentiated phenotype, consistent with a tumor-suppressive effect of TIF1γ relying on its capacity to inhibit Smad4-dependent EMT in late stage of tumorigenesis. Today, the exact mechanism by which TIF1γ behaves as a tumor suppressor independently of Smad4 in the early phase of transformation is still unclear, and an active tumor- suppressive role of TIF1γ in late stages of tumorigenesis that would constrain Smad4-induced EMT still needs to be demonstrated. The following model can be proposed to illustrate the complex role of TIF1γ in cancer (Fig. 6.2).

6.3 Conclusion

Genetically engineered mouse models bearing mutations found in human tumors have largely contributed to demonstrating in vivo the tumor-suppressive role of TGF-β. They have also provided crucial mechanistic information by revealing the crucial role of TGF-β-induced programs such as apoptosis and senescence in preventing transformation. These models have provided precious information on the capacity of TGF-β to limit progression of pre-neoplastic lesions towards aggressive tumors. By combining TGF-β signaling mutations with other mutations found in human tumors, it has been possible to generate mouse models that better mimic human diseases. These models have also enlightened the crucial role played by the microenvironment during carcinogenesis and have provided powerful systems to explore the origin of cancer cells. Finally, one can envision that these models will represent valuable tools for preclinical studies, an obligate step to develop new anticancer treatments.

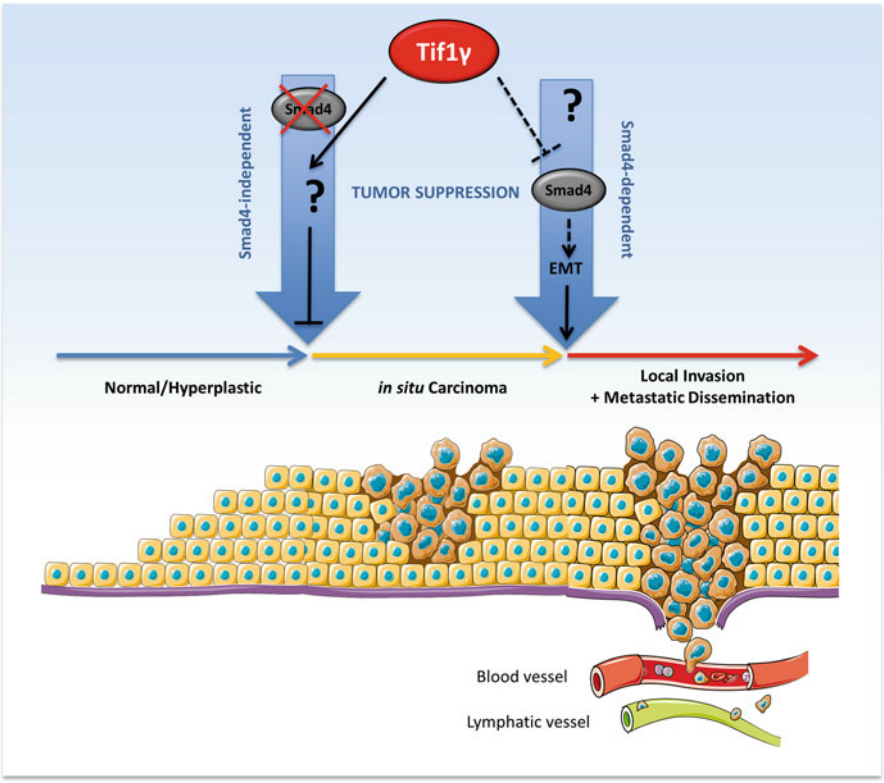

Fig. 6.2 Tumor-suppressive effects of TIF1γ. *Note*: The figures were illustrated using Servier Medical Art

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