Mouse models of transforming growth factor β impact in breast development and cancer

R Serra and M R Crowley

Department of Cell Biology, University of Alabama at Birmingham, 1918 University Boulevard, Birmingham, AL 35294-0005, USA

(Requests for offprints should be addressed to R Serra; Email: rserra@uab.edu)

(Present address of M R Crowley is Department of Genetics, University of Alabama, Birmingham, 720 20th Street South, Birmingham, AL 35294-0024, USA)

Abstract

It is now recognized that transforming growth factor β (TGF- β) is an important factor that regulates normal breast development as well as breast cancer. Genetically engineered mouse models have been used to determine the role and mechanism of TGF- β action in normal development and diseases of the breast. Using these models, it has been determined that TGF- β regulates many steps of normal mammary gland development including branching morphogenesis, functional differentiation, cell-lineage decisions, and involution. Effects of TGF- β on normal development are mediated through signaling in both the epithelial and stromal compartments. In cancer, mouse models have indicated that TGF- β has biphasic effects on tumor progression, acting as a tumor suppressor in early stages of cancer and promoting invasion and metastasis at later stages. In addition, TGF- β may play a role in tumor progression through effects on the microenvironment. Recently, experiments in several mouse models have suggested that antagonism of TGF- β signaling may provide a therapeutic target for late-stage breast cancer, blocking metastasis without detrimental side effects. In the future, genetically altered mice will be used to establish models of human breast disease providing opportunities to test strategies for disease prevention and treatment.

Endocrine-Related Cancer (2005) 12 749–760

Introduction

Development of the mouse mammary gland begins at embryonic day 11 (E11) as an invagination of the ectoderm into the underlying ventral mesoderm (Imagawa *et al.* 1994, Hennighausen & Robinson 2001, Silberstein 2001, Parmar & Cunha 2004). A prominent mammary bud surrounded by condensed mammary mesenchyme is evident by E15. The mammary bud elongates slightly into the primitive mammary fat pad resulting in a mammary anlagen that consists of 12–15 branches at birth. The anlagen grows isometrically with the mouse until puberty at which time ovarian hormones stimulate the formation of large terminal end buds (TEBs) that rapidly invade the fat pad. Eventually, the TEBs reach the limits of the fat pad and regress. The adult female mammary gland then undergoes limited rounds of proliferation,

differentiation, and apoptosis in response to hormonal changes of the estrus cycle. Pregnancy induces the differentiation of alveolar milk producing cells and lobuloalveolar structures along the existing ductal tree that eventually fill the fat pad with epithelium. Lactation signals a fully functional mammary gland and the differentiated cells are capable of producing and secreting milk. After weaning, milk stasis results in a process called involution. Involution is a two-step process that first involves programmed cell death of the milk-producing cells followed by extensive matrix remodeling, returning the mammary gland to a quiescent state. Many of the highly orchestrated biological processes that occur during the cycle of normal mammary gland development, including cell growth, invasion, differentiation, and apoptosis, are deregulated during the formation and progression of mammary cancer.

The transforming growth factor β (TGF- β) superfamily is recognized as a large and evolutionarily conserved family of secreted multifunctional peptides involved in regulating almost every aspect of cellular behavior (Miller *et al.* 1990, Roberts & Sporn 1990, Kingsley 1994, Massague *et al.* 2000). TGF- β is the prototype for this large peptide family. Members of the superfamily include three isoforms of TGF- β (TGF- β 1, TGF- β 2, and TGF- β 3): the activins and inhibins; the growth and differentiation factors (GDFs); and the bone morphogenetic proteins (BMPs). TGF- β is first synthesized as a precursor protein with a signal sequence and a large prodomain (Pircher *et al.* 1986). TGF- β is secreted in an inactive, latent form consisting of a 25 kDa mature peptide in a non-covalent association with the N-terminal prodomain of the precursor protein (latency-associated peptide; Wakefield *et al.* 1989). Activation of the latent form of TGF- β may be a major regulatory step in controlling TGF- β function *in vivo* (Barcellos-Hoff 1996, Murphy-Ullrich & Poczatek 2000, Koli *et al.* 2001). A constitutively active form of TGF- β can be produced by mutations in cysteine residues 223 and 225 (Tgfb^{223/225}) that abrogate the ability of latency-associated peptide to associate with the mature peptide (Brunner *et al.* 1989).

TGF- β signals through a heterotetrameric receptor complex composed of type I (T β RI) and type II (T β RII) serine threonine kinases (Fig. 1; Derynck 1994, Ten Dijke *et al.* 1996, Massague 1998b). TGF- β ligand binds to T β RII on the cell surface and recruits T β RI into the heterotetrameric complex (Wrana *et al.* 1994). T β RII, a constitutively active kinase, phosphorylates and activates the kinase function of T β RI. Downstream substrates of T β RI transduce the signal to the nucleus. The most well-characterized TGF- β signaling molecules are the Smads (Derynck & Zhang 1996, Massague 1998a, Massague & Wotton 2000, Wrana & Attisano 2000). Receptor-associated Smads (R-Smads) interact with and are directly phosphorylated by T β RI. Phosphorylated R-Smads can associate with Smad4, translocate to the nucleus, and regulate transcription either alone or in combination with transcriptional co-activators and co-repressors from other signaling cascades. In this way TGF- β signaling is integrated with signaling from other growth factors (Zhang & Derynck 1999, Attisano & Wrana 2000, Massague & Chen 2000). Specific R-Smads transduce distinct signals for members of the TGF- β superfamily. Smad2 and Smad3 mediate signaling by TGF- β and activin whereas Smad1, Smad5, and Smad8 mediate BMP signaling. Inhibitory Smads (I-Smads), Smad6 and Smad7, have also been identified (Hayashi *et al.* 1997, Hata *et al.* 1998). TGF- β receptors and Smads

Figure 1 TGF- β signaling pathway. TGF- β binds to the type II receptor (II) on the cell surface. The type II receptor associates with and phosphorylates the type I receptor (I). The type I receptor phosphorylates receptor-associated Smads (R-Smads), which form a complex with Smad4, translocate to the nucleus, and act as transcription factors in combination with a variety of co-factors. Dominant-negative forms of the type II receptor lack kinase activity, preventing phosphorylation and activation of type I receptor and subsequent signaling events.

have been identified as potential tumor-suppressor genes in humans (Markowitz *et al.* 1995, Eppert *et al.* 1996, Hahn *et al.* 1996, Serra & Moses 1996, Massague *et al.* 2000, Derynck *et al.* 2001).

Mouse models of TGF- β in mammary development

Members of the TGF- β superfamily, their receptors, and signaling molecules are expressed and play critical roles in every phase of post-natal mammary gland development (reviewed in Daniel *et al.* 2001 and Serra & Crowley 2004). *In situ* hybridization analysis of developing mouse mammary glands with probes

Table 1 Genetically engineered mouse models to determine the role of TGF- β signaling in breast development and disease

Gene Alteration	Strain name	Reference
Ligand		
Gain of function	MMTV-Tgfb1 ^{223/225} WAP-Tgfb1 ^{223/225} β -Lac-Tgfb3 ^{223/225} TetOp-Tgfb1 ^{223/225}	Pierce <i>et al.</i> 1993 Jhappan <i>et al.</i> 1993 Nguyen & Pollard, 2000 Muraoka-Cook <i>et al.</i> 2004
Loss of function	Tgfb1 ^{+/-} Tgfb3 ^{-/-}	Ewan <i>et al.</i> 2002 Nguyen & Pollard, 2000
Receptors		
Gain of function	MMTV-T β RI ^{AAV}	Siegel <i>et al.</i> 2003
Loss of function	MMTV-DNIIR MT-DNIIR MMTV-T β RII-AS T β RII ^{MMTVcreKO} T β RII ^{FSP1creKO} MMTV-Fc: T β RII	Bottinger <i>et al.</i> 1997 Gorska <i>et al.</i> 1998 Siegel <i>et al.</i> 2003 Joseph <i>et al.</i> 1999 Lenferink <i>et al.</i> 2003 Forrester <i>et al.</i> 2005 Cheng <i>et al.</i> 2005 Yang <i>et al.</i> 2002b
Smad		
Loss of function	Smad3 ^{-/-} Smad4 ^{MMTVcreKO}	Yang <i>et al.</i> 2002a Li <i>et al.</i> 2003

specific for the different TGF- β isoforms demonstrated overlapping patterns of expression in the TEBs as well as the growth-quiescent ducts (Robinson *et al.* 1991). Expression levels were increased during pregnancy but dropped dramatically during lactation (Robinson *et al.* 1991). The message for TGF- β 3 increased 6 h after the cessation of lactation (Nguyen & Pollard 2000) followed by increases in TGF- β 1 and - β 2 that peaked between 4 and 6 days of involution (Faure *et al.* 2000, Strange *et al.* 1992). Localization of TGF- β proteins in the mouse mammary gland has also been characterized (reviewed in Ewan *et al.* 2002). TGF- β proteins were localized in distinct and overlapping patterns in both epithelial and stromal compartments. T β RI and T β RII have also been localized in the mouse by *in situ* hybridization and immunohistochemistry to both the epithelial and stromal compartments during all phases of mammary gland development (Joseph *et al.* 1999).

The expression pattern of TGF- β s in the mouse suggested that TGF- β could have roles in regulating branching morphogenesis, lactation, and involution. Experiments using mouse models have confirmed a role for TGF- β in several steps of post-natal mammary gland development (Table 1; Fig. 2). The growth-suppressive effects of TGF- β on the TEBs were first demonstrated by implantation of slow-release pellets containing active TGF- β 1 or TGF- β 3 in the mammary fat pad in front of the elongating ductal tree (Silberstein & Daniel 1987, Robinson *et al.* 1991). Whole-mount and histological analysis of control

mammary glands implanted with pellets containing BSA showed large, multilayered TEBs with high rates of proliferation. In contrast, the TEBs in glands implanted with beads containing TGF- β regressed and had proliferation rates similar to quiescent mature ducts (Silberstein & Daniel 1987, Robinson *et al.* 1991). The effects of TGF- β were reversible. Transgenic mice that expressed the active form of TGF- β in mammary epithelium under the control of the mouse mammary tumor virus (MMTV) promoter, MMTV-Tgfb1^{223/225}, demonstrated a hypoplastic mammary gland (Pierce *et al.* 1993). That is, the ductal epithelium was delayed in filling the fat pad and virtually devoid of side branches (Pierce *et al.* 1993). Proliferation rates were reduced in the mammary epithelium 2-fold at 7 weeks and 4-fold at 13 weeks in the transgenic gland compared with wild-type animals (Pierce *et al.* 1993). Conversely, mice heterozygous for a null allele of the TGF- β 1 gene (*Tgfb1*^{+/-}) have 10% of the TGF- β 1 protein observed in wild-type mice. *Tgfb1*^{+/-} mice demonstrated increased ductal extension through the fat pad and increased proliferation rates of both end-bud and ductal epithelium (Ewan *et al.* 2002). Mice with a dominant-negative interference of T β RII function also demonstrated increased ductal extension through the end bud (Crowley *et al.* 2005a). The data suggest that TGF- β normally acts as an inhibitor of ductal elongation and branching. In contrast, mammary glands from *Smad3*-null mice demonstrated delayed ductal development and decreased side

Figure 2 Role of TGF- β in mouse mammary development. TGF- β inhibits progression of the end buds through the fat pad during puberty (A), regulates side branching via the stroma (B), prevents formation of alveolar buds and premature milk secretion (C), and promotes apoptosis during involution (D).

branching, most likely due to impaired ovarian function (Yang *et al.* 2002a). Post-natal development was normal in *Smad3*-null mice supplied with exogenous hormones, suggesting that signaling through *Smad3* is not inherently necessary for normal mammary gland development.

Stromal-epithelial communication is critical for proper mammary gland development (Neville *et al.* 1998, Parmar & Cunha 2004). The stroma, composed of fibroblasts, adipocytes, lymphocytes, and other cell types, provides instructive signals that regulate epithelial behavior. Advances in manipulating mouse genetics combined with sophisticated surgical techniques have lead to the ability to evaluate signaling between stromal and epithelial compartments in the mammary gland. Several proteins, including estrogen receptor, epidermal growth factor receptor, inhibin, gelsolin, and c-Cbl, exert their effects on epithelial branching morphogenesis through the mammary stroma (Cunha *et al.* 1997, Robinson & Hennighausen 1997, Wiesen *et al.* 1999, Crowley *et al.* 2000, 2005). Mice that express a dominant-negative form of T β RII under the control of the metallothionein promoter (MT-DNIIR) demonstrated increased ductal side branching (Joseph *et al.* 1999). Reciprocal transplantation experiments indicated that the side-branching phenotype was mediated through the stroma (Crowley *et al.* 2005). These data, in association with loss of TGF- β 1 staining at developing side branches, suggest that stromal TGF- β signaling is an important regulator of ductal side branching in the mammary gland (Ewan *et al.* 2002). In contrast, conditional deletion of T β RII in fibroblasts using the Cre/Lox recombinaase system, where Cre was under the control of the FSP1 promoter (T β RII^{FspKO}), resulted in reduced ductal morphogenesis and end-bud formation (Cheng *et al.* 2005). Defects were also observed in the composition of the stroma, with a reduction in the proportion of adipocytes to fibroblasts. Since the FSP1 promoter was activated during embryonic development, differentiation of cells in the fat pad could have been

affected. It was shown previously that fat tissue is required for normal ductal outgrowth (Gregoire 2001, Couldrey *et al.* 2002), thus it is not clear whether the results in the conditional knockout are a direct effect of TGF- β signaling in the periductal stroma or an indirect effect of alterations in mesenchymal differentiation.

TGF- β also regulates the development and survival of alveolar epithelium. Mice in which active TGF- β is expressed under the control of the pregnancy-specific whey acid protein promoter (WAP-Tgfb1^{223/225}) displayed normal branching morphogenesis but the development of secretory epithelium was inhibited as a result of early apoptotic death in the differentiating alveolar cells (Jhappan *et al.* 1993, Kordon *et al.* 1995, Smith 1996). Similarly, transgenic mice expressing activated T β RI in the mammary epithelium (MMTV-T β RI^{AD}) displayed deficiencies in late pregnancy due to increased apoptosis in alveolar structures (Siegel *et al.* 2003). Conversely, dominant-negative interference of TGF- β signaling in the mammary epithelium (MMTV-DNIIR) resulted in precocious alveolar development; that is, virgin mice demonstrated an early pregnant phenotype (Bottinger *et al.* 1997, Gorska *et al.* 1998). Since the phenotype was not detected until the mice were 20 weeks of age, the alveolar development seen in these mice most likely required several rounds of proliferation and apoptosis that normally occur during the estrus cycle. Accumulation of alveolar cells could be a consequence of a failure of the cells to be removed through apoptosis (Gorska *et al.* 1998). Crosses of MMTV-DNIIR and *prolactin*-null mice indicated that precocious alveolar development in the MMTV-DNIIR mice was dependent on prolactin (Bailey *et al.* 2004). It was suggested that prolactin and TGF- β co-ordinate alveolar development through careful regulation of apoptosis mediated by Akt. Inappropriate alveolar development has also been observed in mice expressing T β RII antisense mRNA and in mice with Cre/Lox-mediated conditional deletion of T β RII in mammary epithelium (T β RII^{MMTVKO}) (Lenferink *et al.* 2003, Forrester *et al.*

2005). Likewise, the reduced levels of TGF- β 1 found in *Tgfb1*^{+/-} mice resulted in an increase in alveolar development during pregnancy (Ewan *et al.* 2002). The data together suggest that TGF- β acts to limit inappropriate alveolar development and to regulate alveolar outgrowth during pregnancy. TGF- β 's ability to regulate apoptosis is likely to be involved.

Post-lactational milk stasis triggers the first wave of apoptosis in secretory alveoli during involution. High levels of TGF- β 3 mRNA were detected in mammary gland 6 h after the induction of milk stasis (Nguyen & Pollard 2000). Transgenic mice that expressed TGF- β 3 under the control of the β -lactoglobulin promoter, which directs expression to alveolar epithelial cells in late pregnancy and throughout lactation, showed a significant increase in apoptotic cells at days 1 and 3 of involution compared with control, non-transgenic mice. Increased apoptosis was concomitant with Smad4 nuclear translocation and induction of phosphorylated Stat 3 (Nguyen & Pollard 2000). In addition, when mammary glands from TGF- β 3-null mice, which die a few days after birth, were transplanted into syngeneic wild-type hosts, a decrease in alveolar apoptosis compared with heterozygous or wild-type glands was observed during involution (Nguyen & Pollard 2000). Furthermore, dominant-negative interference of T β R II in mammary epithelium (MMTV-DNIIR) results in a delay in involution in response to weaning or sealing the duct (Gorska *et al.* 2003, Bailey *et al.* 2004). The levels of phosphorylated Akt and Forkhead transcription factor 01 (FKHR) were increased in MMTV-DNIIR mice compared with wild-type controls, suggesting that TGF- β may act to restrain Akt and FKHR activity thereby promoting apoptosis during the initial stages of involution (Bailey *et al.* 2004). Together the results suggest that TGF- β 3 signaling through T β R II has a key role in the early phase of involution. TGF- β 3 may also play a role in re-organization of the extracellular matrix in the late stage of involution. Faure *et al.* (2000) determined that the primary site of TGF- β 3 synthesis in late involution was myoepithelial cells. Expression corresponded to sites of laminin deposition leading the authors to propose that TGF- β 3 was involved in stromal reorganization (Faure *et al.* 2000).

In addition to accelerated apoptosis in differentiating cells, mammary epithelial cells from WAP-Tgfb1^{223/225} mice demonstrated another phenotype, early growth senescence as seen by a diminished capacity of epithelial cells to repopulate a cleared fat pad (Kordon *et al.* 1995). Recently, a new parity-induced population of epithelial cells was discovered in the mouse mammary gland (Wagner *et al.* 2002,

Boulanger *et al.* 2005). This population of cells is derived from differentiating cells during pregnancy and was identified by marking WAP-expressing cells with a *lacZ* reporter gene using Cre/Lox recombinase technology. It was shown that *lacZ*-positive cells accumulated in the mammary gland after pregnancy but did not accumulate in nulliparous mice with normal estrus cycle. The lack of persistence of *lacZ*-positive cells in nulliparous mice suggested that cells that differentiated during estrus were removed, most likely through apoptosis as described above. In contrast, a population of cells induced during pregnancy were maintained and contributed to future rounds of differentiation. The parity-induced cell population was shown to be pluripotent and capable of self-renewal (Boulanger *et al.* 2005). Experiments with mice expressing activated TGF- β 1 in the Cre/Lox/Lac Z background suggested that TGF- β 1 limited the self-renewal capacity of this cell population. The discovery of the parity-induced population of cells indicates that not all differentiated cells are removed during involution and explains some of the biological differences between nulliparous and parous glands.

Recently, Smad4, a central mediator of TGF- β signaling, was deleted in mammary gland epithelium using a Cre/Lox recombination strategy (Li *et al.* 2003). Early mammary gland development was normal; however, lack of Smad4 induced gradual increases in proliferation, alveolar differentiation, and transdifferentiation of mammary epithelium into squamous cells. Mice later developed mammary abscesses and squamous cell carcinoma. The results suggest a role for TGF- β in regulating cell-fate decisions in the mammary gland. In addition, it has been documented that 7,12-dimethylbenz(a)anthracene (DMBA) treatment in mice results in the formation of a heterogeneous group of tumors, the most common being those with squamous metaplasia or adenomyoepithelial character (Cardiff *et al.* 1988, Rehm 1990, Bottinger *et al.* 1997). When mice expressing a dominant-negative form of T β R II were treated with DMBA, alterations in TGF- β signaling influenced the pathology of the tumors formed (Bottinger *et al.* 1997, Crowley M, Frost A, Chen D-T, Baffi M, Nicola T & Serra R, unpublished work). In wild-type mice, DMBA promoted the formation of adenomyoepitheliomas. This type of tumor was not detected in glands with altered TGF- β signaling. Furthermore, staining for α SMA and K14 was reduced or absent in glandular structures from transgenic tumors relative to wild-type tumors (Crowley M, Frost A, Chen D-T, Baffi M, Nicola T & Serra R, unpublished work). The data suggest that TGF- β is also involved in the differentiation, maintenance, and/or transformation of myoepithelial cells.

Mouse models of TGF- β in breast cancer

The growth-suppressive effects of TGF- β on epithelial cells are well documented (Alexandrow & Moses 1995). Analysis of *T β RI* and *T β RII* genes in colon and gastric cancers with microsatellite instability (RER+, replication error) provided genetic evidence of a tumor-suppressor role for TGF- β (Markowitz *et al.* 1995). In addition, several genes that encode TGF- β signaling molecules, *T β RI* and *T β RII*, *Smad2*, *Smad3*, and *Smad4*, have been found mutated or deleted in a variety of human cancers (Markowitz *et al.* 1995, Serra & Moses 1996, Massague *et al.* 2000, Derynck *et al.* 2001). Furthermore, mRNA levels for TGF- β signaling molecules are altered in many additional tumors. Mutations in the TGF- β signaling pathway are rare in human breast cancer; however, loss of the *T β RII* has been documented by immunohistochemistry (Gobbi *et al.* 1999, 2000). Significant loss of *T β RII* immunoreactivity in epithelial hyperplasia lacking atypia (EHLA) was correlated with an approximate 4-fold increase in relative risk for developing invasive breast cancer (Gobbi *et al.* 1999). Additionally, loss of *T β RII* staining was inversely correlated with tumor grade, mitotic index, and clinical stage (Gobbi *et al.* 2000). Several studies using transgenic mice support a role for TGF- β as a tumor suppressor in the mammary gland. Expression of transforming growth factor- α (TGF- α) in mouse mammary epithelial cells via the MMTV promoter resulted in ductal hyperplasia that progressed to carcinoma (Matsui *et al.* 1990). Co-expression of TGF- β 1 in the mammary epithelium of the MMTV-TGF α mice significantly reduced the number of tumors that formed (Pierce *et al.* 1995). In addition, TGF- β expression alone (MMTV-Tgfb1^{223/225}) inhibited mammary tumor formation in mice challenged with DMBA as compared with DMBA-treated wild-type controls (Pierce *et al.* 1995). In complimentary studies, dominant-negative interference of *T β RII* in mouse mammary epithelium (MMTV-DNIIR) increased the incidence and number of tumors that formed after treatment with DMBA (Bottinger *et al.* 1997). MMTV-DNIIR mice also developed spontaneous tumors with long latency (Gorska *et al.* 2003). Mice that express activated neu in the mammary epithelium (MMTV-neu) are a well-characterized model for mammary tumor development and progression (Muller *et al.* 1988). Mice develop adenocarcinomas that metastasize to the lung in a defined period of time. Mice in which the activated form of *T β RI* was expressed in the mammary epithelium of MMTV-neu mice developed fewer tumors with greater latency than

the MMTV-neu controls (Siegel *et al.* 2003). Conversely, expression of a dominant-negative form of *T β RII* in MMTV-neu mice decreased the latency of tumor onset (Siegel *et al.* 2003). Conditional deletion of *T β RII* in mammary epithelium (*T β RII*^{MMTVKO}) also resulted in shortened tumor latency in the context of polyomavirus middle-T induced oncogenesis (Forrester *et al.* 2005). The data suggest TGF- β s can act as tumor suppressors in breast cancer but the mechanisms are still not clear.

One proposed mechanism involves TGF- β induced senescence in the mammary stem cell population (Kordon *et al.* 1995, Boulanger & Smith 2001). Inoculation of mammary glands with MMTV normally results in the development of hyperplastic alveolar nodules that presumably progress to form cancer in mice (Boulanger & Smith 2001). Introduction of MMTV into the mammary glands of 3–5-month-old WAP-Tgfb1^{223/225} mice showed a decrease in tumor incidence (5.8%) compared with control mice (51.7%). Epithelial cells isolated from WAP-Tgfb1^{223/225}-expressing mammary glands also have a reduced capacity to repopulate cleared mammary fat pads compared with cells from wild-type littermates, suggesting premature senescence of stem cells in the TGF- β -overexpressing tissue (Kordon *et al.* 1995). Recently, a parity-induced, pluripotent, self-renewing population of cells was discovered in the mammary gland (Wagner *et al.* 2002). TGF- β also diminished the self-renewing capability of this cell population, raising the possibility that TGF- β may induce premature senescence in stem cells, thereby reducing the number of target cells for development of cancer (Boulanger *et al.* 2005).

Although there is considerable evidence indicating TGF- β functions as a tumor suppressor, there is also evidence pointing to a role for TGF- β in promoting the progression of cancer and metastasis. Many breast cancers synthesize and secrete high levels of TGF- β protein that can be detected in both the tumor cells and associated stroma (Teicher 2001). Clinical reports suggest that elevated levels of TGF- β in tumor tissue or serum of cancer patients is associated with poor prognosis (Sheen-Chen *et al.* 2001, Teicher 2001, Ivanovic *et al.* 2003). It is thought that several variables, such as tumor stage, affect the way tumor cells respond to TGF- β (Dumont & Arteaga 2000, Wakefield *et al.* 2000). Cells in early-stage tumors respond to TGF- β with a growth-inhibitory response, suppressing further progression of the tumor. Later, as the tumor progresses, a different genetic or epigenetic environment exists and responsiveness to TGF- β is altered so that the tumor-promoting activities of TGF- β dominate. The tumor-promoting functions of TGF- β

can be a result of autocrine, paracrine, or tumor–host interactions (Dumont & Arteaga 2000). Cellular functions affected include regulation of extracellular matrix, angiogenesis, and immuno surveillance as well as epithelial to mesenchymal conversion.

The biphasic role of TGF- β in mammary tumor formation *in vivo* can be seen in transgenic mice that express either activated or dominant-negative forms of the TGF- β ligands and receptors. When expressed under the control of the MMTV promoter, an activated form of T β RI (MMTV-T β RI^{AAV}) suppressed Neu-induced tumorigenesis but promoted metastasis in the tumors that formed (Siegel *et al.* 2003). In contrast, expression of the activated ligand (MMTV-Tgfb1^{223/225}) did not affect tumor latency; however, tumors were more invasive and lung metastasis was more prevalent (Muraoka *et al.* 2003). Inducible expression of active TGF- β 1 (TetOp-Tgfb1^{223/225}) for as little as 2 weeks also increased lung metastasis in mice with polyomavirus middle-T-induced tumors, suggesting that TGF- β can rapidly promote metastatic progression (Muraoka-Cook *et al.* 2004). Conversely, expression of a dominant-negative form of T β RII under the control of the MMTV promoter reduced the incident of metastasis in the MMTV-neu background (Siegel *et al.* 2003). Conditional deletion of T β RII in mammary epithelial cells using Cre/Lox recombination (T β RII^{MMTVKO}) also resulted in decreased latency in polyomavirus middle-T-induced tumors (Forrester *et al.* 2005). Unexpectedly, increased metastasis to the lung was observed in this mouse model (Forrester *et al.* 2005). Several possibilities exist for the discrepancy: incomplete deletion of T β RII in tumor cells, timing for the alterations in TGF- β signaling, or cell-autonomous and non-cell-autonomous actions of TGF- β . Tobin *et al.* (2002) showed increased lung metastasis of MDA-231 cells in response to TGF- β irrespective of the expression of a dominant-negative receptor on the tumor cells, suggesting a role in metastasis that is paracrine-based. Nevertheless, the data suggest TGF- β is an important factor in regulating invasion and metastasis, although the exact mechanism is not clear.

Whereas it has previously been established that normal development of the mammary ductal system requires signals generated from the stroma, only recently has evidence accumulated to suggest that the stroma and microenvironment directly influence the initiation, promotion, or progression of cancer (Noel & Foidart 1998, Tlsty 2001, Tlsty & Hein 2001, Tuxhorn *et al.* 2001, Wiseman & Werb 2002, Erickson & Barcellos-Hoff 2003). Desmoplasia, fibroblast proliferation in conjunction with remodeling of the

extracellular matrix, is often observed in human breast cancers (Noel & Foidart 1998, Erickson & Barcellos-Hoff 2003). This altered stroma is also known as the reactive stroma and has some characteristics in common with granulation tissue found during wound repair (Tuxhorn *et al.* 2001). It has been proposed that TGF- β expressed by cancer cells stimulates the formation of reactive stroma. TGF- β induces myofibroblast differentiation, extracellular matrix remodeling, and angiogenesis, properties of the reactive stroma that could promote the progression of tumors (Tuxhorn *et al.* 2001). Recent experimental studies in mice have implicated TGF- β signaling in the stroma as a regulator of tumor development. When epithelium-free mouse fat pads were exposed to gamma irradiation, tumor development was stimulated in a non-tumorigenic cell line, COMMA-D, suggesting that changes in the stromal environment contribute to neoplastic progression (Barcellos-Hoff & Ravani 2000). Before that, it was shown that gamma irradiation resulted in rapid remodeling of the extracellular matrix and activation of latent TGF- β in the stroma (Barcellos-Hoff 1993, Barcellos-Hoff *et al.* 1994). Furthermore, neutralizing antibodies directed to TGF- β suppressed matrix remodeling in response to irradiation, suggesting a role for activated TGF- β and the remodeled extracellular matrix in neoplastic progression (Ehrhart *et al.* 1997). Subsequently, it was shown through conditional deletion of T β RII in fibroblasts via a FSP1-cre transgene (T β RII^{FSP1KO}) that TGF- β signaling in the stroma modulates oncogenic potential of prostate and stomach epithelium (Bhowmick *et al.* 2004). Mammary cancer could not be investigated in this mouse model since the mice died at around 8 weeks of age. To access the role of TGF- β signaling to the stroma in mammary cancer, co-cultures of polyomavirus middle-T-induced mammary carcinoma cells and T β RII-positive or T β RII-deleted (T β RII^{FSP1KO}) fibroblasts were transplanted under the kidney capsules of nude mice (Cheng *et al.* 2005). T β RII^{FSP1KO} fibroblasts promoted tumor cell growth and invasion, which correlated with increased activation of several tyrosine kinase receptors including c-Met. Furthermore, conditioned medium from T β RII^{FSP1KO} fibroblasts contained high levels of Hepatocyte Growth Factor (HGF) and other growth factors and inhibitors of the growth factors blocked increased cell growth and motility in response to the conditioned media. The results suggest that TGF- β promotes mammary cancer via regulation of various growth factors in the stroma (Cheng *et al.* 2005). The studies together suggest that TGF- β mediates changes in the stroma that contribute to neoplastic progression.

Since TGF- β regulates invasion in mammary tumors, it has the potential to act as a therapeutic target in a clinical setting (Akhurst 2002). Clinically, tumors are generally identified at later stages of progression where the tumor-suppressive actions of TGF- β have little effect. In this regard, the concept of using soluble antagonists of TGF- β to block metastasis is appealing. It has been shown that intraperitoneal injection of a soluble chimeric protein composed of the extracellular portion of T β RII and the Fc portion of IgG heavy chain (Fc:T β RII) blocks tumor metastasis in three different mouse models of breast cancer (Muraoka *et al.* 2002). Tumor cell motility and extravasation as well as matrix metalloproteinase activity were inhibited in the presence of Fc:T β RII. In addition, apoptosis in cancer cells was increased but alterations in tumor cell proliferation were not observed. Furthermore, vascular density within tumors was not affected by treatment with soluble Fc:T β RII. Transgenic mice that expressed Fc:T β RII under the control of the MMTV promoter were also resistant to metastasis compared with wild-type mice in both a tail vein metastasis assay and in crosses with MMTV-neu mice (Yang *et al.* 2002b). Most importantly, mice expressing Fc:T β RII did not demonstrate an increase in the formation of primary tumors and they did not demonstrate adverse side effects even though high levels of Fc:T β RII were detected in the serum. The data support the feasibility of using soluble receptors as injectable drugs in the treatment of breast cancer with few adverse effects.

Summary

Genetically engineered mice have been used to determine the role and mechanism of TGF- β signaling in normal breast physiology and pathology. DNA-regulatory elements that direct the expression of activated TGF- β ligands and receptors as well as dominant-negative forms of various signaling molecules to specific cell types and developmental stages within the mammary gland have lead to an understanding of how TGF- β regulates spatial and temporal aspects of mammary gland development. Sophisticated transplantation techniques in combination with mouse transgenic and knockout models have permitted a discussion of the importance of stromal-epithelial interaction in both normal development and tumor progression. Recent advances using Cre/Lox technology in mice have allowed conditional deletion of genes in specific tissues at specific times, providing additional information regarding TGF- β action in breast development and disease. This information is currently

being used to develop strategies to combat breast cancer via the development of TGF- β antagonists (Muraoka-Cook *et al.* 2005). In the future, these mouse models will allow further testing of additional cancer prevention and treatment strategies.

Acknowledgements

R S is supported by NIH R01CA91974. M R C was supported by DMAD17-01-1-0201. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

- Akhurst RJ 2002 TGF-beta antagonists: why suppress a tumor suppressor? *Journal of Clinical Investigation* **109** 1533–1536.
- Alexandrow MG & Moses HL 1995 Transforming growth factor β and cell cycle regulation. *Cancer Research* **55** 1452–1457.
- Attisano L & Wrana JL 2000 Smads as transcriptional co-modulators. *Current Opinion in Cell Biology* **12** 235–243.
- Bailey JP, Nieport KM, Herbst MP, Srivastava S, Serra RA & Horseman ND 2004 Prolactin and transforming growth factor-beta signaling exert opposing effects on mammary gland morphogenesis, involution, and the Akt-forkhead pathway. *Molecular Endocrinology* **18** 1171–1184.
- Barcellos-Hoff MH 1993 Radiation-induced transforming growth factor β and subsequent extracellular matrix reorganization in murine mammary gland. *Cancer Research* **53** 3880–3886.
- Barcellos-Hoff MH 1996 Latency and activation in the control of TGF-beta. *Journal of Mammary Gland Biology and Neoplasia* **1** 353–363.
- Barcellos-Hoff MH & Ravani SA 2000 Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. *Cancer Research* **60** 1254–1260.
- Barcellos-Hoff MH, Derynck R, Tsang ML-S & Weatherbee JA 1994 Transforming growth factor- β activation in irradiated murine mammary gland. *Journal of Clinical Investigation* **93** 892–899.
- Bhowmick NA, Chytil A, Plith D, Gorska AE, Dumont N, Shappell S, Washington MK, Neilson EG & Moses HL 2004 TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* **303** 848–851.
- Bottinger EP, Jakubczak JL, Haines DC, Bagnall K & Wakefield LM 1997 Transgenic mice overexpressing a dominant-negative mutant type II TGF- β receptor show enhanced tumorigenesis in the mammary gland and lung in response to the carcinogen 7,12-dimethylbenz[*a*]-anthracene. *Cancer Research* **57** 5564–5570.

- Boulanger CA & Smith GH 2001 Reducing mammary cancer risk through premature stem cell senescence. *Oncogene* **20** 2264–2272.
- Boulanger CA, Wagner KU & Smith GH 2005 Parity-induced mouse mammary epithelial cells are pluripotent, self-renewing and sensitive to TGF- β 1 expression. *Oncogene* **24** 552–560.
- Brunner AM, Marquardt H, Malacko AR, Lioubin MN & Purchio AF 1989 Site-directed mutagenesis of cysteine residues in the pro region of the transforming growth factor β -1 precursor. *Journal of Biological Chemistry* **264** 13660–13664.
- Cardiff RD, Gumerlock PH, Soong MM, Dandekar S, Barry PA, Young LJ & Meyers FJ 1988 c-H-ras-1 expression in 7,12-dimethyl benzantracene-induced Balb/c mouse mammary hyperplasias and their tumors. *Oncogene* **3** 205–213.
- Cheng N, Bhowmick NA, Chytil A, Gorska AE, Brown KA, Muraoka R, Arteaga CL, Neilson EG, Hayward SW & Moses HL 2005 Loss of TGF- β type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through upregulation of TGF- α , MSP-, and HGF-mediated signaling networks. *Oncogene* **24** 5053–5068.
- Couldrey C, Moitra J, Vinson C, Anver M, Nagashima K & Green J 2002 Adipose tissue: a vital in vivo role in mammary gland development but not differentiation. *Developmental Dynamics* **223** 459–468.
- Crowley MR, Head KL, Kwiatkowski DJ, Asch HL & Asch BB 2000 The mouse mammary gland requires the actin-binding protein gelsolin for proper ductal morphogenesis. *Developmental Biology* **225** 407–423.
- Crowley M, Bowtell DD & Serra R 2005a TGF- β , c-Cbl, and PDGFR α in the mouse mammary stroma. *Developmental Biology* **279** 58–72.
- Cunha GR, Young P, Hom YK, Cooke PS, Taylor JA & Lubahn DB 1997 Elucidation of a role for stromal steroid hormone receptors in mammary gland growth and development using tissue recombinants. *Journal of Mammary Gland Biology and Neoplasia* **2** 393–402.
- Daniel CW, Robinson S & Silberstein GB 2001 The transforming growth factors β in development and functional differentiation of the mouse mammary gland. *Advanced Experimental Medical Biology* **501** 61–70.
- Derynck R 1994 TGF- β -receptor-mediated signaling. *Trends in Biochemical Science* **19** 548–553.
- Derynck R & Zhang Y 1996 Intracellular signaling: The MAD way to do it. *Current Biology* **6** 1226–1229.
- Derynck R, Akhurst RJ & Balmain A 2001 TGF- β signaling in tumor suppression and cancer progression. *Nature Genetics* **29** 117–129.
- Dumont N & Arteaga CL 2000 Transforming growth factor- β and breast cancer: Tumor promoting effects of transforming growth factor- β . *Breast Cancer Research* **2** 125–132.
- Ehrhart EJ, Segarini P, Tsang ML, Carroll AG & Barcellos-Hoff MH 1997 Latent transforming growth factor β 1 activation in situ: quantitative and functional evidence after low-dose gamma-irradiation. *FASEB Journal* **11** 991–1002.
- Eppert K, Scherer SW, Ozcelik H, Pirone R, Hoodless P, Kim H, Tsui LC, Bapat B, Gallinger S, Andrulis IL et al. 1996 MADR2 maps to 18q21 and encodes a TGF- β regulated MAD-related protein that is functionally mutated in colorectal carcinoma. *Cell* **86** 543–552.
- Erickson AC & Barcellos-Hoff MH 2003 The not-so innocent bystander: the microenvironment as a therapeutic target in cancer. *Expert Opinion in Therapeutic Targets* **7** 71–88.
- Ewan KB, Shyamala G, Ravani SA, Tang Y, Akhurst R, Wakefield L & Barcellos-Hoff MH 2002 Latent transforming growth factor- β activation in mammary gland: regulation by ovarian hormones affects ductal and alveolar proliferation. *American Journal of Pathology* **160** 2081–2093.
- Faure E, Heisterkamp N, Groffen J & Kaartinen V 2000 Differential expression of TGF- β isoforms during postlactational mammary gland involution. *Cell Tissue Research* **300** 89–95.
- Forrester E, Chytil A, Bierie B, Aakre M, Gorska AE, Sharif-Afshar AR, Muller WJ & Moses HL 2005 Effect of conditional knockout of the type II TGF- β receptor gene in mammary epithelia on mammary gland development and polyomavirus middle T antigen induced tumor formation and metastasis. *Cancer Research* **65** 2296–2302.
- Gobbi H, Dupont WD, Simpson JF, Plummer WD Jr, Schuyler PA, Olson SJ, Arteaga CL & Page DL 1999 Transforming growth factor- β and breast cancer risk in women with mammary epithelial hyperplasia. *Journal of the National Cancer Institute* **91** 2096–2101.
- Gobbi H, Arteaga CL, Jensen RA, Simpson JF, Dupont WD, Olson SJ, Schuyler PA, Plummer WD Jr & Page DL 2000 Loss of expression of transforming growth factor β type II receptor correlates with high tumour grade in human breast in-situ and invasive carcinomas. *Histopathology* **36** 168–177.
- Gorska AE, Joseph H, Derynck R, Moses HL & Serra R 1998 Dominant-negative interference of the transforming growth factor β type II receptor in mammary epithelium results in alveolar hyperplasia and differentiation in virgin mice. *Cell Growth and Differentiation* **9** 229–238.
- Gorska AE, Jensen RA, Shyr Y, Aakre ME, Bhowmick NA & Moses HL 2003 Transgenic mice expressing a dominant-negative mutant type II transforming growth factor- β receptor exhibit impaired mammary development and enhanced mammary tumor formation. *American Journal of Pathology* **163** 1539–1549.
- Gregoire FM 2001 Adipocyte differentiation: from fibroblast to endocrine cell. *Experiments in Biological Medicine (Maywood)* **226** 997–1002.
- Hahn SA, Schutte M, Hoque ATMS, Moskaluk CA, Da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH et al. 1996 *DPC4*, a candidate tumor

- suppressor gene at human chromosome 18q21.1. *Science* **271** 350–353.
- Hata A, Lagna G, Massague J & Hemmati-Brivanlou A 1998 Smad6 inhibits BMP/Smad1 signaling by specifically competing with the Smad4 tumor suppressor. *Genes and Development* **12** 186–197.
- Hayashi H, Abdollah S, Qiu Y, Cai J, Xu YY, Grinnell BW, Richardson MA, Topper JN, Gimbrome MA Jr, Wrana JL et al. 1997 The MAD-related protein Smad7 associates with the TGF β receptor and functions as an antagonist of TGF β signaling. *Cell* **89** 1165–1173.
- Hennighausen L & Robinson GW 2001 Signaling pathways in mammary gland development. *Developments in Cell* **1** 467–475.
- Imagawa W, Yang J, Guzman R & Satybrata N 1994 Control of mammary gland development. In *The Physiology of Reproduction*, 2nd edn, pp 1033–1063. Eds E Knobil & JD Neil. New York: Raven Press.
- Ivanovic V, Todorovic-Rakovic N, Demajo M, Neskovic-Konstantinovic Z, Subota V, Ivanisevic-Milovanovic O & Nikolic-Vukosavljevic D 2003 Elevated plasma levels of transforming growth factor-beta 1 (TGF-beta 1) in patients with advanced breast cancer: association with disease progression. *European Journal of Cancer* **39** 454–461.
- Jhappan C, Geiser AG, Kordon EC, Bagheri D, Hennighausen L, Roberts AB, Smith GH & Merlino G 1993 Targeting expression of a transforming growth factor β 1 transgene to the pregnant mammary gland inhibits alveolar development and lactation. *EMBO Journal* **12** 1835–1845.
- Joseph H, Gorska A, Sohn P, Moses HL & Serra R 1999 Overexpression of a kinase-deficient transforming growth factor- β type II receptor in mouse mammary stroma results in increased epithelial branching. *Molecular Biology of the Cell* **10** 1221–1234.
- Kingsley DM 1994 The TGF- β superfamily: New members, new receptors, and new genetic tests of function in different organisms. *Genes and Development* **8** 133–146.
- Koli K, Saharinen J, Hyytiainen M, Penttinen C & Keski-Oja J 2001 Latency, activation, and binding proteins of TGF-beta. *Microscopic Research Techniques* **52** 354–362.
- Kordon EC, McKnight RA, Jhappan C, Hennighausen L, Merlino G & Smith GH 1995 Ectopic TGF β 1 expression in the secretory mammary epithelium induces early senescence of the epithelial stem cell population. *Developments in Biology* **168** 47–61.
- Lenferink AE, Magoon J, Pepin MC, Guimond A & O'Connor-McCourt MD 2003 Expression of TGF-beta type II receptor antisense RNA impairs TGF-beta signaling in vitro and promotes mammary gland differentiation in vivo. *International Journal of Cancer* **107** 919–928.
- Li W, Qiao W, Chen L, Xu X, Yang X, Li D, Li C, Brodie SG, Meguid MM, Hennighausen L et al. 2003 Squamous cell carcinoma and mammary abscess formation through squamous metaplasia in Smad4/Dpc4 conditional knockout mice. *Development* **130** 6143–6153.
- Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B et al. 1995 Inactivation of the type II TGF- β receptor in colon cancer cells with microsatellite instability. *Science* **268** 1336–1338.
- Massague J 1998a TGF- β signal transduction. *Annual Review of Biochemistry* **67** 753–791.
- Massague J 1998b TGF- β signal transduction. *Annual Review of Biochemistry* **67** 753–791.
- Massague J & Chen YG 2000 Controlling TGF-beta signaling. *Genes & Development* **14** 627–644.
- Massague J & Wotton D 2000 Transcriptional control by the TGF-beta/Smad signaling system. *EMBO Journal* **19** 1745–1754.
- Massague J, Blain SW & Lo RS 2000 TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell* **103** 295–309.
- Matsui Y, Halter SA, Holt JT, Hogan BLM & Coffey RJ Jr 1990 Development of mammary hyperplasia and neoplasia in MMTV-TGFalpha transgenic mice. *Cell* **61** 1147–1156.
- Miller DA, Pelton RW, Derynck R & Moses HL 1990 Transforming growth factor- β : A family of growth regulatory peptides. *Annals of the New York Academy of Sciences* **593** 208–217.
- Muller WJ, Sinn E, Pattengale PK, Wallace R & Leder P 1988 Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. *Cell* **54** 105–115.
- Muraoka RS, Dumont N, Ritter CA, Dugger TC, Brantley DM, Chen J, Easterly E, Roebuck LR, Ryan S, Gotwals PJ et al. 2002 Blockade of TGF-beta inhibits mammary tumor cell viability, migration, and metastases. *Journal of Clinical Investigation* **109** 1551–1559.
- Muraoka RS, Koh Y, Roebuck LR, Sanders ME, Brantley-Sieders D, Gorska AE, Moses HL & Arteaga CL 2003 Increased malignancy of Neu-induced mammary tumors overexpressing active transforming growth factor beta1. *Molecular and Cell Biology* **23** 8691–8703.
- Muraoka-Cook RS, Kurokawa H, Koh Y, Forbes JT, Roebuck LR, Barcellos-Hoff MH, Moody SE, Chodosh LA & Arteaga CL 2004 Conditional overexpression of active transforming growth factor beta1 in vivo accelerates metastases of transgenic mammary tumors. *Cancer Research* **64** 9002–9011.
- Muraoka-Cook RS, Dumont N & Arteaga CL 2005 Dual role of transforming growth factor beta in mammary tumorigenesis and metastatic progression. *Clinical Cancer Research* **11** 937s–943s.
- Murphy-Ullrich JE & Poczatek M 2000 Activation of latent TGF-beta by thrombospondin-1: mechanisms and physiology. *Cytokine Growth Factor Reviews* **11** 59–69.
- Neville MC, Medina D, Monks J & Hovey RC 1998 The mammary fat pad. *Journal of the Mammary Gland Biology and Neoplasia* **3** 109–116.

- Nguyen AV & Pollard JW 2000 Transforming growth factor beta3 induces cell death during the first stage of mammary gland involution. *Development* **127** 3107–3118.
- Noel A & Foidart JM 1998 The role of stroma in breast carcinoma growth in vivo. *Journal of the Mammary Gland Biology and Neoplasia* **3** 215–225.
- Parmar H & Cunha GR 2004 Epithelial-stromal interactions in the mouse and human mammary gland in vivo. *Endocrine-related Cancer* **11** 437–458.
- Pierce DF Jr, Johnson MD, Matsui Y, Robinson SD, Gold LI, Purchio AF, Daniel CW, Hogan BLM & Moses HL 1993 Inhibition of mammary duct development but not alveolar outgrowth during pregnancy in transgenic mice expressing active TGF- β 1. *Genes and Development* **7** 2308–2317.
- Pierce DF Jr, Gorska AE, Chytil A, Meise KS, Page DL, Coffey RJ Jr & Moses HL 1995 Mammary tumor suppression by transforming growth factor β 1 transgene expression. *PNAS* **92** 4254–4258.
- Pircher R, Jullien P & Lawrence DA 1986 Beta-transforming growth factor is stored in human blood platelets as a latent high molecular weight complex. *Biochemical and Biophysical Research Communications* **136** 30–37.
- Rehm S 1990 Chemically induced mammary gland adenomyoepitheliomas and myoepithelial carcinomas of mice. Immunohistochemical and ultrastructural features. *American Journal of Pathology* **136** 575–584.
- Roberts AB & Sporn MB 1990 The transforming growth factor- β s. In *Peptide Growth Factors and their Receptors*, pp 419–472. Eds MB Sporn & AB Roberts. Heidelberg: Springer-Verlag.
- Robinson GW & Hennighausen L 1997 Inhibin and activins regulate mammary epithelial cell differentiation through mesenchymal-epithelial interactions. *Development* **124** 2701–2708.
- Robinson SD, Silberstein GB, Roberts AB, Flanders KC & Daniel CW 1991 Regulated expression and growth inhibitory effects of transforming growth factor- β isoforms in mouse mammary gland development. *Development* **113** 867–878.
- Serra R & Crowley M 2004 TGF- β in mammary gland development and breast cancer. *Breast Disease* **18** 61–73.
- Serra R & Moses HL 1996 Tumor suppressor genes in the TGF- β signaling pathway? *Nature Medicine* **2** 390–391.
- Sheen-Chen SM, Chen HS, Sheen CW, Eng HL & Chen WJ 2001 Serum levels of transforming growth factor beta1 in patients with breast cancer. *Archives in Surgery* **136** 937–940.
- Siegel PM, Shu W, Cardiff RD, Muller WJ & Massague J 2003 Transforming growth factor {beta} signaling impairs Neu-induced mammary tumorigenesis while promoting pulmonary metastasis. *PNAS* **100** 8430–8435.
- Silberstein GB 2001 Postnatal mammary gland morphogenesis. *Microscope Research Techniques* **52** 155–162.
- Silberstein GB & Daniel CW 1987 Reversible inhibition of mammary gland growth by transforming growth factor-beta. *Science* **237** 291–293.
- Smith GH 1996 TGF-beta and functional differentiation. *Journal of the Mammary Gland Biology and Neoplasia* **1** 343–352.
- Strange R, Li F, Saurer S, Burkhardt A & Friis RR 1992 Apoptotic cell death and tissue remodelling during mouse mammary gland involution. *Development* **115** 49–58.
- Teicher BA 2001 Malignant cells, directors of the malignant process: role of transforming growth factor-beta. *Cancer Metastasis Reviews* **20** 133–143.
- Ten Dijke P, Miyazono K & Heldin C-H 1996 Signaling via hetero-oligomeric complexes of type I and type II serine treonine kinase receptors. *Current Opinion in Cell Biology* **8** 139–145.
- Tlsty TD 2001 Stromal cells can contribute oncogenic signals. *Seminars in Cancer Biology* **11** 97–104.
- Tlsty TD & Hein PW 2001 Know thy neighbor: stromal cells can contribute oncogenic signals. *Current Opinion in Genetic Development* **11** 54–59.
- Tobin SW, Douville K, Benbow U, Brinckerhoff CE, Memoli VA & Arrick BA 2002 Consequences of altered TGF-beta expression and responsiveness in breast cancer: evidence for autocrine and paracrine effects. *Oncogene* **21** 108–118.
- Tuxhorn JA, Ayala GE & Rowley DR 2001 Reactive stroma in prostate cancer progression. *Journal of Urology* **166** 2472–2483.
- Wagner KU, Boulanger CA, Henry MD, Sgagias M, Hennighausen L & Smith GH 2002 An adjunct mammary epithelial cell population in parous females: its role in functional adaptation and tissue renewal. *Development* **129** 1377–1386.
- Wakefield LM, Smith DM, Broz S, Jackson M, Levinson AD & Sporn MB 1989 Recombinant TGF-beta 1 is synthesized as a two-component latent complex that shares some structural features with the native platelet latent TGF-beta 1 complex. *Growth Factors* **1** 203–218.
- Wakefield LM, Yang YA & Dukhanina O 2000 Transforming growth factor-beta and breast cancer: Lessons learned from genetically altered mouse models. *Breast Cancer Research* **2** 100–106.
- Wiesen JF, Young P, Werb Z & Cunha GR 1999 Signaling through the stromal epidermal growth factor receptor is necessary for mammary ductal development. *Development* **126** 335–344.
- Wiseman BS & Werb Z 2002 Stromal effects on mammary gland development and breast cancer. *Science* **296** 1046–1049.
- Wrana JL & Attisano L 2000 The Smad pathway. *Cytokine Growth Factor Reviews* **11** 5–13.
- Wrana JL, Attisano L, Wieser R, Ventura F & Massagué J 1994 Mechanism of activation of the TGF- β receptor. *Nature* **370** 341–347.
- Yang YA, Tang B, Robinson G, Hennighausen L, Brodie SG, Deng CX & Wakefield LM 2002a Smad3 in the mammary epithelium has a nonredundant role in the induction of apoptosis, but not in the regulation of

- proliferation or differentiation by transforming growth factor-beta. *Cell Growth & Differentiation* **13** 123–130.
- Yang YA, Dukhanina O, Tang B, Mamura M, Letterio JJ, MacGregor J, Patel SC, Khozin S, Liu ZY, Green J et al. 2002b Lifetime exposure to a soluble TGF-beta antagonist protects mice against metastasis without adverse side effects. *Journal of Clinical Investigation* **109** 1607–1615.
- Zhang Y & Derynck R 1999 Regulation of Smad signalling by protein associations and signalling crosstalk. *Trends in Cell Biology* **9** 274–279.