

Review

Tumour–stromal interactions

Transforming growth factor- β isoforms and hepatocyte growth factor/scatter factor in mammary gland ductal morphogenesis

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Abstract

The mammary gland undergoes morphogenesis through the entire reproductive life of mammals. In mice, ductal outgrowth from the nipple across the fat pad results in an intricate, well spaced ductal tree that further ramifies and develops alveolar structures during pregnancy. Ductal morphogenesis is regulated by the concerted action of circulating steroid and polypeptide hormones, and local epithelial–mesenchymal inductive signals. Transforming growth factor (TGF)- β_{1-3} and hepatocyte growth factor (HGF)/scatter factor (SF) are important components of this latter signaling pathway. TGF- β_1 and TGF- β_3 have roles in both promotion and inhibition of branching morphogenesis that are dependent on concentration and context. HGF/SF promotes ductal outgrowth and tubule formation in the mammary gland. These data suggest that these two growth factors have complementary roles in promoting mammary ductal morphogenesis and in maintaining ductal spacing. In addition, TGF- β_3 triggers apoptosis in the alveolar epithelia, which is a necessary component of mammary gland involution and return of the ductal structure to a virgin-like state after lactation.

Keywords: apoptosis, ductal morphogenesis, growth factor, mammary gland, SMAD

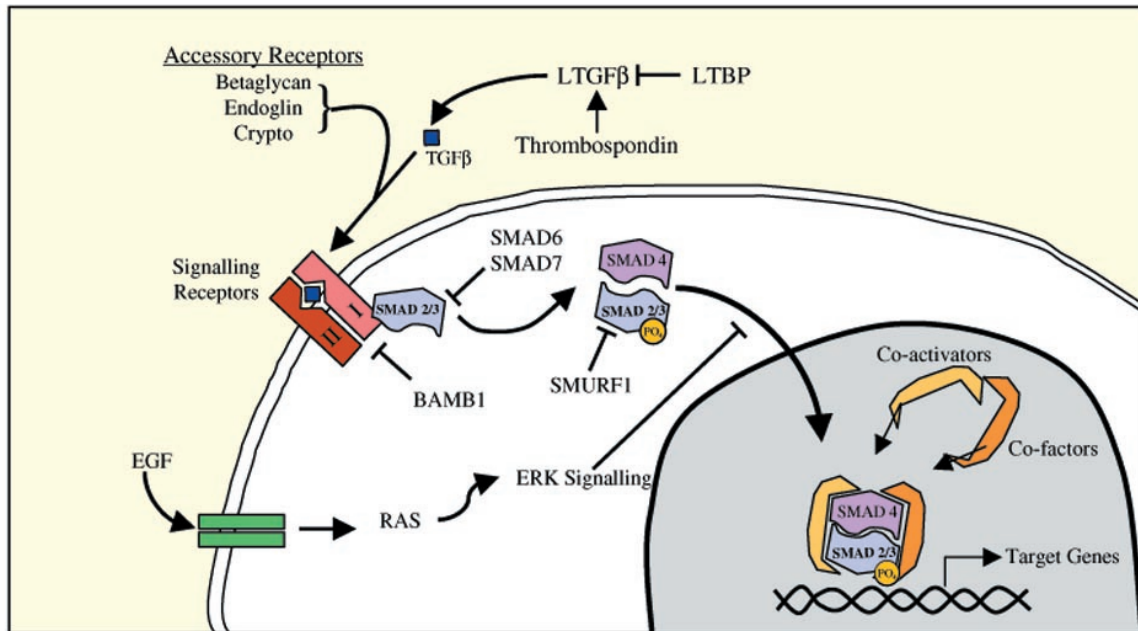
Introduction

The development of the mammary gland largely occurs postnatally. Initially, the ductal system begins to develop from the nipple, and is characterized by specialized structures – the terminal end buds (TEBs) – capping the end of the ducts. TEBs grow out across the fat pad and, by bifurcation, lay down by puberty the minimally branched structure that covers the fat pad, at which point the TEBs disappear. Throughout the estrus cycle there is modest development of the ductal system, but it is during pregnancy that a dramatic outgrowth of secondary branches

occurs that, together with the formation of the lobuloalveolar structures, results in the milk-producing gland. Once weaning has occurred, this fully developed structure regresses to a virgin-like state.

It has been well established that these events are under the control of a complex interplay of circulating hormones, particularly estrogen, progesterone, glucocorticoids and prolactin gene family members [1]. However, it has also become clear that local mesenchymal–epithelial interactions are essential for mammary development, and that

Figure 1



Schematic of the TGF- β signaling pathway, showing the activation cascade and points of inhibition (\perp). Active TGF- β , released from the latent complex by the action of thrombospondin, binds to the type II receptor, resulting in the formation of an active receptor–signaling complex. This binding is enhanced by accessory receptors, whereas the receptor dimerization can be inhibited by membrane-bound inhibitors such as bone morphogenic protein and activin membrane bound inhibitor (BAMBI). After ligand binding, the receptor SMADs are phosphorylated, dimerize with SMAD-4, and translocate to the nucleus where they recruit appropriate cofactors and coactivators to stimulate transcription of target genes. TGF- β signaling can be blocked by the inhibitory SMADs, SMAD-6 and SMAD-7, which prevent receptor–SMAD activation; and by the ras pathway, which can lead to inhibition of nuclear translocation of the dimeric SMAD complex. Receptor SMADs can also be degraded following ubiquitylation by the ubiquitin ligase SMURF1. Figure adapted from Massagué [5].

many of these are mediated by growth factors that are often synthesized in response to the circulating hormones. These include wnt family proteins, TGF- α , fibroblast growth factors, insulin-like growth factors, epidermal growth factor [2], colony stimulating factor-1 [3], and – the subjects of the present review – the TGF- β family of proteins and HGF/SF.

Transforming growth factor- β isoforms

The three classical members of the TGF- β family belong to a much larger family. In humans this family contains almost 30 members, including bone morphogenic proteins, activins, and Mullerian inhibiting substance [4]. These TGF- β family members have profound effects during development, regulating cell fate by affecting proliferation, differentiation and cell death, and therefore they are important for the development of many tissues.

All three of the classical TGF- β isoforms (TGF- β s) activate intracellular responses by binding to and heterodimerizing the same two transmembrane serine/threonine kinases (Fig. 1) [5]. In this complex the type II receptor phosphorylates the type I receptor (also known as activin receptor-like kinase), activation of which results in propagation of the

signal intracytoplasmically to the transcription machinery. This propagation is via the direct phosphorylation of SMAD proteins [6]. Eight SMAD family members have been documented. In the case of TGF- β s, however, SMAD-2 and SMAD-3 (or receptor phosphorylated SMADs) are the positive signaling SMADs. These phosphorylated SMADs heterodimerize with a common shared partner, SMAD-4. After this they translocate to the nucleus where, by recruitment of coactivator proteins, they participate in the transcriptional activation of target genes. In contrast, SMAD-6 and SMAD-7 are negative regulators. SMAD-7 binds to the TGF- β -activated receptor, where it inhibits the phosphorylation of SMAD-2 and SMAD-3, consequently downregulating the signaling pathway. SMAD-7 expression is also upregulated by TGF- β ₁, allowing it to act in an autoinhibitory feedback loop that shuts off TGF- β signaling. Negative control also occurs by degradation of SMADs following their ubiquitylation by SMURF-1 [5].

There is also growing evidence for other postreceptor signaling pathways (probably involving the mitogen-activated protein kinase pathway) and for other cell-type specific TGF- β receptors [6]. Furthermore, several intracellular sig-

naling pathways interact with the TGF- β signaling pathway to ensure cell-type specific responses through activation of different genes. Discussion of this aspect of TGF- β biology is beyond the scope of the present review, and the reader is referred to the several recent excellent reviews on this topic [4,5,7].

In order to understand the roles of TGF- β *in vivo*, it is also important to appreciate that there are several extracellular controls on TGF- β function that can spatially and temporally regulate TGF- β action to allow context dependent action (Fig. 1). Most significantly, TGF- β s are synthesized as latent inactive precursors of transforming growth factor (LTGF)- β , consisting of the 24-kDa cytokine and a 80-kDa dimer of the prepro region called the latency-associated protein. During secretion of TGF- β s from the cell the latency-associated protein region is cleaved from the precursor molecule by Furin-mediated proteolysis, followed by a noncovalent attachment to the dimeric TGF- β . In nontransformed cells, LTGF- β is covalently bound to LTGF- β binding proteins (of which four distinct ones have been described) and deposited in the extracellular matrix. LTGF- β is inactive, and therefore activation is a key mechanism in the action of TGF- β ; this activation is stimulated by proteases, with the majority of *in vivo* activation ascribed to thrombospondin-1 [8]. In addition to the availability of active TGF- β , its signaling is also regulated by membrane anchored coreceptors such as β -glycan (also called TGF- β type III receptor), endoglin and crypto, which potentiate the binding of TGF- β to its receptor. Thus, *in vivo*, a complex web of interacting proteins exerts both negative and positive controls over the action of TGF- β .

Transforming growth factor- β isoforms in the mammary gland

Expression patterns of transforming growth factor- β isoforms in the mammary gland

In the mammary gland TGF- β s are expressed in a complex and overlapping pattern [9], suggesting important roles for them in its development. All three TGF- β s are expressed in the epithelium of the TEBs, although TGF- β_2 has a very low expression at this stage, and throughout the rest of ductal development. In most ductal areas, expressions of TGF- β_1 and TGF- β_3 are overlapping. However, TGF- β_1 is found uniquely at the tips of the developing bud, whereas TGF- β_3 transcripts are found in the flanking region.

These patterns of mRNA expression are largely recapitulated by protein expression, although most studies did not distinguish active from latent TGF- β s. Preliminary studies were conducted by Barcellos-Hoff and Ewan [10], who used antibodies that can discriminate active from latent TGF- β_1 ; those investigators showed mosaic expression of active and inactive TGF- β_1 in the mammary epithelium, whereas only LTGF- β was found in the stroma. TGF- β_3 expression was also found uniquely in the myoepithelial cells, whereas TGF- β_1 was deposited in the stroma around quiescent terminal ducts.

During pregnancy, TGF- β_1 is expressed at low levels in alveolar and ductal structures, whereas TGF- β_2 has a lower but similar pattern of expression. TGF- β_3 is the most strongly expressed of all the isoforms in the alveolar and ductal structures. Toward the end of pregnancy and during lactation, all isoforms are heavily downregulated [9]. On weaning, however, TGF- β_3 mRNA and protein is dramatically upregulated in the alveolar epithelial and ductal cells [11,12].

TGF- β receptor II expression is found in the ductal epithelial and surrounding stromal cells in virgin, pregnant, and involuting mammary gland. Similarly, *in situ* hybridization localized TGF- β receptor I (activin receptor-like kinase 5) to the same cells at all these stages of development [13], suggesting that both epithelial and mesenchymal cells can respond to endogenous TGF- β .

Modes of action of transforming growth factor- β isoforms in the mammary gland

Experiments that have introduced TGF- β s into the mammary gland by implantation or via transgenic means have largely suggested that TGF- β_1 is a growth inhibitor, suppressing ductal morphogenesis (Table 1). The first experiments of this type employed the implantation of slow-release pellets containing TGF- β s into the mammary fat pad [14]. All three TGF- β isoforms suppressed TEB formation (in a reversible manner), causing the ends of the ducts to take on the appearance of growth-quiescent ducts [9]. Expression of constitutively active TGF- β_1 from the mouse mammary tumor virus (MMTV) promoter resulted in a hypoplastic ductal tree, but with no effect on lobuloalveolar development [15]. In contrast, TGF- β_1 expressed from the later acting whey acidic protein promoter inhibited alveolar, but not ductal development during pregnancy [16]. This was associated with a reduction in the stem cell pool available for lobuloalveolar development [17].

More recently transgenic techniques have been employed to express dominant-negative forms of the TGF- β receptor, which are aimed at inhibition of all TGF- β isoform signaling in the mammary gland. Expression of such a dominant-negative construct in the mammary epithelium from the MMTV promoter resulted in precocious alveolar hyperplasia in virgin mice, but without any effect on TEB formation [18]. Expression of the same construct primarily in mammary stromal cells, however, caused an increase in lateral branching, suggesting that these stromal cells were the primary sites of action of epithelial expressed TGF- β s [13]. This is consistent with observations that TGF- β_1 slow-release implants in cleared fat pads suppressed the formation of new end buds in transplanted mammary epithelium [19]. Together, these experiments are in line with roles for TGF- β_1 and TGF- β_3 in the maintenance of ductal spacing by inhibition of lateral branching through epithelial-mesenchymal signaling (Fig. 2).

Table 1**Genetic analysis of TGF- β and HGF/SF function in the mammary gland**

Experimental design	Cell type expression*/tissue affected†	Phenotype	Reference
Gain of function			
MMTV-TGF- β_1	Mammary epithelium	Inhibition of ductal development	[15]
WAP-TGF- β_1	Lobuloalveolar progenitors and cells	Inhibition of alveolar development; aging of stem cells	[17]
β -lactoglobulin-TGF- β_3	Alveolar and ductal epithelial cells during lactation	Induction of apoptosis	[12]
Retroviral HGF	Primary mammary epithelial cells transplanted into the mammary fat pad	Increased TEB number and branching	[32]
Loss of function			
MMTV-dnTGF- β receptor II	Mammary epithelium	Precocious lobuloalveolar development and milk production	[18]
MMTV-dnTGF- β receptor II	Mammary stroma	Increased lateral branching	[13]
TGF- $\beta_1^{-/-}$ SCID	Whole animal	Hypoplastic ductal tree	Ingram W, Robertson SA, personal communication
TGF- $\beta_1^{+/-}$ p21 $^{-/-}$	Whole animal	Accelerated mammary development; normal adult structure	[10]
SMAD-3 $^{-/-}$	Whole animal	Hypoplastic mammary epithelium	[21]
TGF- $\beta_3^{-/-}$	Mammary epithelium into wild-type stroma	Reduced apoptosis during involution. Normal mammary development	[12]
dnMet	Mammary tumor cells in culture followed by transplantation to mice	Increased tubule formation	[30]

*For gain of function. †For loss of function. dn, dominant negative. MMTV, mouse mammary tumor virus; WAP = whey acidic protein.

Insights gained from transforming growth factor- β gene knockout mice

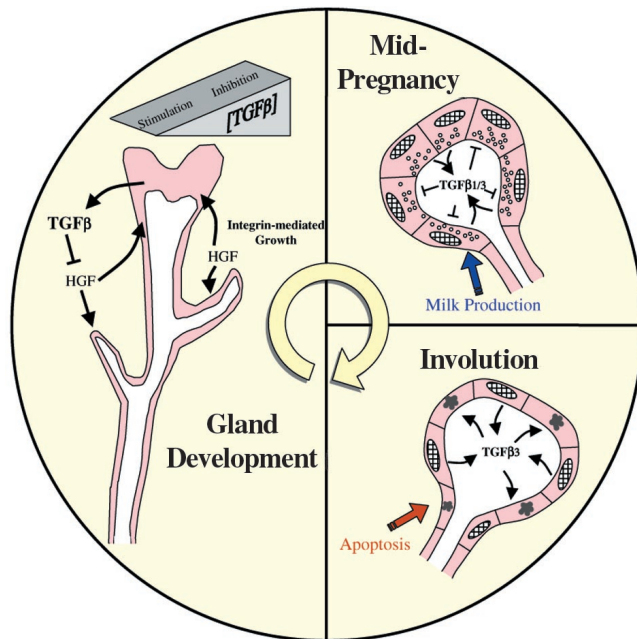
Considerable insights into biologic processes have been gained by the generation of null mutations in mice (Table 1). All three TGF- β genes have been mutated, as have their receptors, several SMAD genes, and LTGF- β binding proteins [20]. Generally, these experiments were uninformative regarding mammary development. Perhaps the greatest insights might have come from targeted ablation of the SMAD genes. Unfortunately, SMAD-4- and SMAD-2-null mutant mice die very early in gestation, precluding mammary gland studies. However, SMAD-3 $^{-/-}$ mice survive up to eight weeks of age. Detailed analysis of mammary development has yet to be reported [20], although a preliminary analysis [21] suggested that the mammary glands are under-developed.

The TGF- β_1 -null phenotype is variable, with many mice dying during embryogenesis due to vasculature defects, although in some cases they die within a few days of life because of multifocal inflammatory lesions. However, these mice can be maintained if they are crossed with severe combined immunodeficiency (SCID) mice [20], or with mice that are nullizygous for the cell cycle inhibitor p21 [21]. Preliminary analysis of mammary development in

TGF- $\beta_1^{-/-}$ SCID mice shows a dramatic reduction in ductal complexity, suggesting an essential role for TGF- β_1 in ductal outgrowth (Ingman WV, Robertson SA, personal communication). Interestingly, on the p21 $^{-/-}$ background, TGF- $\beta_1^{+/-}$ mice (in which TGF- β_1 concentrations are significantly reduced) show accelerated mammary development [10]. TGF- β_3 -null mutant mice also die at birth, but transplantation of their rudimentary mammary glands into recipient SCID mice allowed mammary development to be assessed. This did not reveal any gross defects in branching morphogenesis, however, and the glands apparently developed normally through to lactation [12].

At first sight these results on the mammary gland development of TGF- β_1 -null and TGF- β_3 -null mutant mice are difficult to reconcile with the experiments described above suggesting inhibitory roles for TGF- β s in the branching morphogenesis of this organ. However, in cultures of TAC-2.1 mammary cells, low concentrations of TGF- β_1 had a positive effect on tubule and branching formation that was coincident with the inhibition of colony formation, whereas high concentrations only inhibited colony formation and had no effect on morphogenesis. Low concentrations also stimulated cell proliferation, whereas high doses inhibited this parameter, an effect

Figure 2



Postulated roles for TGF- β and HGF/SF during the life cycle of the mammary gland. TGF- β s have complex roles during mammary development, according to their concentration. Although most of the experimental evidence points to inhibition of ductal branching, preliminary analysis of both TGF- β_1 - and SMAD-3-null mutant mice suggest a requirement for TGF- β_1 for mammary development. Thus, TGF- β s have both concentration- and context-dependent effects on mammary gland development. HGF/SF promotes ductal outgrowth through an integrin-mediated process. This action is inhibited by TGF- β_1 , indicating that interactions between these growth factors play a role in ductal spacing as the mammary gland grows out over the fat pad. During pregnancy, TGF- β s appear to inhibit alveolar development and suppress milk formation. The decline in TGF- β expression at the end of pregnancy appears to be necessary for full lobuloalveolar development and lactation. After weaning involution is begun, with TGF- β_3 inducing apoptosis in the lobuloalveolar structures through an autocrine mechanism.

that was dependent on the extracellular matrix [22]. Thus, TGF- β s might promote the outgrowth of TEBs and lateral branches at low concentration, whereas higher concentrations suppress growth in terminal end ducts and along the ductal length, perhaps by inhibiting expression or action of HGF/SF, as has been reported in culture [23] (Fig. 2). These conclusions would be consistent with the opposite effects on mammary development observed in mice with one or two mutant alleles of TGF- β_1 . Confirmation of this interpretation will await detailed studies on the mammary glands of the homozygous and heterozygous mutants. This by necessity will involve mammary gland transplantation in order to confirm that the effects of the mutations are organ autonomous.

Toward the end of pregnancy TGF- β expression is dramatically downregulated, with very low levels of TGF- β_2 mRNA detected during lactation [9,11,12]. Milk protein expression by mammary epithelial cells in culture, in response to lactogenic hormones, is suppressed by TGF- β_1 [24,25]. Expression of a TGF- β receptor II dominant-negative transgene in the mammary epithelium resulted in premature expression of casein [18]. These results, together with those regarding over-expression of TGF- β_1 from the whey acidic protein promoter (Table 1) [18], suggest that another role for TGF- β s is to regulate lobuloalveolar development and suppress milk production during pregnancy. These effects are alleviated by down-regulation of these proteins toward the end of pregnancy, such that the full development of the mammary gland during lactation is permitted (Fig. 2).

Immediately after weaning, expression of TGF- β_3 is dramatically induced in the alveolar epithelium in a pattern that precedes the onset of apoptosis and that persists for several days at a lower, but still significant level [11,12]. Transgenic over-expression of TGF- β_3 during lactation induced alveolar epithelial apoptosis without causing gland involution. TGF- β_3 -null mutant mammary epithelial tissue transplanted into the cleared fat pad of a wild-type recipient developed and underwent lactation, but on weaning the tissue showed a lower rate of apoptosis than that in wild-type control tissue, but without a complete ablation of this response. Analysis of SMAD-4 nuclear association indicated that the alveolar epithelium was the target for TGF- β_3 acting through an autocrine mechanism. This induction of TGF- β_3 was in response to milk stasis rather than changes in the hormonal milieu. Together the data provide compelling evidence that TGF- β_3 is one of the mammary-derived signals that induce apoptosis during the first phase of involution [12] (Fig. 2).

Hepatocyte growth factor/scatter factor

HGF/SF, originally identified by its motogenic activity on cultured cells, is a pleiotropic cytokine that promotes survival and proliferation in a wide variety of cells. It also has dramatic effects on cell morphology, triggering intrinsic morphogenic events such as tubule formation in many epithelial cell lines, including those from the mammary gland. It binds to and signals through a unique transmembrane tyrosine kinase receptor, the product of the c-met proto-oncogene. In general, the c-met oncogene is expressed in epithelial cells, whereas the ligand is expressed in the surrounding mesenchyme, providing a mechanism for the epithelial-mesenchymal inductive processes during development. HGF/SF receptor signals in large part via the ras signaling pathway initially after binding the docking protein Grb2, which is essential for c-met signaling [26]. HGF/SF also binds to the extracellular matrix, and heparan sulfate proteoglycans potentiate HGF/SF signaling [27].

Ductal morphogenesis and branching of the resultant tubules can be induced by HGF/SF in primary and certain established mammary epithelial cells cultured in collagen gels or matrigel [23,28,29]. Expression of a dominant-negative c-met receptor can also reverse the tumorigenic phenotype of DN-DA3 mouse mammary adenocarcinoma cell line after transplantation into nude mice, causing them to adopt a differentiated phenotype that is characterized by extensive tubule formation [30]. HGF/SF is synthesized in the mammary stroma, probably by fibroblasts, and acts on receptor-expressing ductal epithelial cells [23]. Together these experiments strongly suggest that HGF/SF c-met signaling is a classical epithelial–mesenchymal inductive pathway that is important for ductal morphogenesis in the mammary gland (Fig. 2). Despite their dramatic nature, however, a caveat of the culture experiments is that the cells form unilaminar tubes that do not resemble the elaborate TEBs that are the primary growth point and determinant of branching in the developing mammary gland.

Unfortunately, null mutants in both *HGF/SF* and *c-met* genes in mice cause embryonic lethality because of placental insufficiency and developmental defects in the liver and muscle [31]. Over-expression of HGF/SF or c-met in the mammary epithelium resulted in hyperplasia and tumorigenesis, but the effects on early mammary development were not assessed [23]. In order to overcome these problems, Yant *et al* [32] transfected primary mouse mammary epithelial cells with a retrovirus containing HGF/SF cDNA, and transplanted these into a cleared recipient fat pad. This increased TEB size and number, and resulted in a hyperplastic ductal tree. These data are consistent with a positive role for HGF/SF in ductal morphogenesis (Fig. 2).

Conclusion

The exquisitely branched structure of the mammary gland requires sophisticated positional clues to maintain ductal spacing. TEBs, with their unique club-shaped multilaminar structures, are both the growth and branching decision points for the developing mammary gland in mice. These structures are encased in a basement membrane, and during their growth they recruit around their body a unique stroma that includes macrophages, eosinophils, and fibroblasts. Unexpectedly, both macrophages and eosinophils appear to regulate ductal morphogenesis, because this process is inhibited in mouse mutants that reduce the abundance of these cells in the mammary gland [3]. Interestingly, macrophages can both produce and respond to TGF- β s, suggesting that these migratory cells could locally and transiently modify the responsiveness of the tissue to these factors. For example, TGF- β_1 inhibits cytokine induction of matrix metalloproteinase-12 in macrophages [33], possibly limiting their ability to remodel matrix and thereby suppressing lateral branching.

Furthermore, macrophages have been shown to activate LTGF- β_1 following binding to cell surface expressed β -glycan [34]. Thus, it may be speculated that at least part of the role of macrophages in regulating branching morphogenesis is to activate epithelial cell-derived TGF- β_1 and, in turn, to have their remodeling functions modified by TGF- β_1 in a very precise pattern. This is consistent with the role of TGF- β_1 in matrix remodeling after radiation-induced damage of the mammary gland [35].

The epithelial cells of the TEBs also need to migrate through the stroma as they grow out across the fat pad. TGF- β_1 can also reversibly induce trans-differentiation of mammary epithelial cells to mesenchyme-like cells that may be important in the migration of cells during TEB outgrowth [36]. Perturbation of β_1 -integrin signaling by blocking antibodies affects TEB formation and inhibits mammary gland development [37]. Anti- β_1 -integrin antibodies blocked HGF/SF-induced tubulogenesis in cultures of mammary epithelial cells, suggesting that HGF/SF signals to the integrin pathways to promote cellular adhesion during ductal morphogenesis [37]. A major effect of TGF- β s is to modulate matrix deposition by fibroblasts, cells that are found around the TEB. This suggests that TGF- β s and HGF/SF may co-operate in the regulation of the migration of epithelial cells across the fatty stroma by altering integrin–matrix signaling locally. Furthermore, in other tissues TGF- β s play an important role in angiogenesis [38], a process that is assumed to be essential for ductal outgrowth and necessary for mammary gland function. Thus, it is likely that TGF- β s locally regulate angiogenesis to accommodate the developing mammary gland.

In addition to these effects of TGF- β s on the stroma, at probably higher concentrations TGF- β s inhibit ductal development through their cell cycle inhibitory effects on epithelial cells. There is considerable understanding, particularly in mammary epithelial cells in culture, of the intracellular pathways that mediate the growth inhibitory properties of TGF- β_1 . TGF- β_1 maintains the retinoblastoma protein in its cell cycle inhibitory hypophosphorylated state through the induction of the cyclin-dependent inhibitors p15^{ink4b} and p21^{cip1/waf1} and the downregulation of cyclin-dependent kinase 2 phosphatase cdc25A [4]. The early signal transduction pathways via SMAD-2, -3, and -4 are also well documented [4]. In contrast, HGF/SF promotes epithelial cellular proliferation and ductal outgrowth in culture. Its signal transduction pathway through the c-met receptor has also been well studied [31]. In fact, a downstream kinase target, Gab1, of this receptor mimicked the action of HGF/SF on tubule formation when transfected into cultured EpH4 mouse mammary epithelial cells [28].

Most of this information on HGF/SF and TGF- β signal transduction has been derived from cells in culture, however, and has not been extrapolated to, or confirmed

in the mammary gland *in vivo*. Considerable insight could therefore be obtained by analysis of these signaling pathways in the mammary gland during development. This is especially important given the multiple levels of control over TGF- β and HGF action. It is essential to define sites of TGF- β_1 and HGF/SF signaling. A powerful tool in this approach will be use of the immunolocalization of nuclear SMAD-4 and of antiphosphorylation specific anti-c-met antibodies as surrogate markers within cells for TGF- β receptor and c-met activation, respectively. These studies could help define the temporal and spatial requirements in different cell types throughout the development of the mammary gland. Similarly, conditional ablation of specific signaling molecules in particular cell types in the mammary gland could reveal individual functions for these growth factors. Allelic series of mutants can also reveal differential functions, as was elegantly shown for oncogenic signaling during mammary gland cancer [39] and for the role of the Grb2 binding site on the c-met receptor that was specifically required for its function in muscle, but not placental or liver development [31].

HGF/SF and TGF- β s appear to have important roles in branching morphogenesis, lobuloalveolar development, and involution in mice. Similar expression patterns in other species, including human, argue for similar roles in these organisms [40,41]. These growth factors integrate within a complex web of growth factor and hormone signaling to pattern the ductal tree. These include other members of the TGF- β family, such as activins and inhibins [1], as well as other growth factors that influence mammary gland development, such as insulin-like growth factor-1, which has also recently been shown to modulate TGF- β_1 expression in this organ [42]. The complexity of the responses and the multiple levels of regulation, including the presence of latent forms and extracellular binding proteins, suggest that cell culture experiments will be inadequate to tease out the different roles for these proteins. Only sophisticated genetic experiments *in vivo*, coupled with the unique ability of the mammary gland elements to be transplanted between genotypes, can provide causality for the involvement of TGF- β s and HGF/SF in different aspects of mammary gland morphogenesis and function.

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