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IGF Ligand and Receptor Regulation of Mammary Development

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Abstract

The insulin-like growth factors, IGF-I and IGF-II, have endocrine as well as autocrine-paracrine actions on tissue growth. Both IGF ligands are expressed within developing mammary tissue throughout postnatal stages with specific sites of expression in the epithelial and stromal compartments. The elucidation of circulating versus local actions and of epithelial versus stromal actions of IGFs in stimulating mammary epithelial development has been the focus of several laboratories. The recent studies addressing IGF ligand function provide support for the hypotheses that 1) the diverse sites of IGF expression may mediate different cellular outcomes, and 2) IGF-I and IGF-II are distinctly regulated and have diverse functions in mammary development. The mechanisms for IGF function likely are mediated, in part, through diverse IGF signaling receptors. The local actions of the IGF ligands and receptors as revealed through recent publications are the focus of this review.

Keywords

IGF-I; IGF-II; IGF-IR; insulin receptor; epithelial-stromal interactions; autocrine-paracrine; stem/progenitor cells

Introduction

Insulin like-growth factor (IGF)-I and -II are growth factors with demonstrated roles in mammary gland morphogenesis. Unlike many other growth factors, the IGFs have both endocrine as well as autocrine/paracrine actions in tissues (1–4). The IGFs act on cells through binding to the IGF-type I receptor (IGF-IR) and, in the case of IGF-II, also to a splice variant of the insulin receptor (IR) known as the IR-A (5). In addition, the IGFs have high affinity for IGF-IR:IR hybrid receptors (hybridR) (6–8). The IGF-IR has known roles in mammary epithelial development, but the precise roles for the other IGF signaling receptors remains poorly defined. Local expression patterns of IGF-I and IGF-II were reviewed previously in this journal along with studies demonstrating essential functions for IGF-I and the IGF-IR (9,10). The goal of this article is to review data published during the past eight years that have further elucidated function of the IGFs in mammary development with a focus on autocrine/paracrine versus endocrine functions as well as epithelial-stromal IGF functions.

Circulating vs local IGFs in development and tissue growth

IGF-I, IGF-II and the IGF-IR are essential for normal embryogenesis (11,12) and promote proliferation, survival or differentiation of cells in numerous tissues. The traditional view is that growth hormone (GH) induces production of IGF-I by the liver and subsequent endocrine

IGF-I then mediates systemic growth during puberty (13). While the IGFs are abundant in serum they also are expressed locally in most tissues postnatally. Whether circulating or locally produced IGFs impact peripheral tissues has been the focus of numerous studies and is an ongoing area of debate (2,4,14)(see also Lann & LeRoith in this issue). Initial studies that challenged the importance of circulating IGF-I and supported the hypothesis that local tissue expression of the IGFs is sufficient for normal postnatal growth came from studies on mice with a liver-specific deletion of IGF-I (LID). Although LID mice have a 75% decrease in circulating IGF-I, no effect on body growth was observed (15–17). In a subsequent study by Yakar and colleagues, circulating IGF-I was reduced to 10%–15% of normal levels in mice by crossing LID mice with mice carrying a null mutation of the acid labile subunit (ALS), part of the tripartite complex important for prolonging half-life of IGF-I in serum (18). Single gene disrupted mice (LID or ALSKO) showed relatively normal growth whereas mice with disruption of both genes (LID/ALSKO) had significant reduction in growth suggesting that a minimal level of circulating IGF-I is necessary for normal bone growth.

The issue of circulating versus local IGFs in regulating tissue growth postnatally is highly relevant to mammary gland/breast development since the majority of epithelial growth occurs postnatally in both rodents and humans. In particular, ductal elongation and outgrowth are initiated at the onset of puberty coincident with the increase in ovarian hormones and pituitary GH. The initial indication of pubertal ductal growth is the appearance of terminal end buds (TEBs), structures that form at the tips of elongating mammary ducts that drive outgrowth and branching of the early ductal structure. The dependence of TEB formation and mammary epithelial growth on GH and its primary target, IGF-I, is well documented and reviewed in this issue by Kleinberg and colleagues. Experiments to dissect the importance of circulating versus mammary-specific IGF-I production were performed by Richards et al utilizing various genetic mutations of the *igf1* gene in mice (19). Since mice with a complete deletion of *igf1* have reduced viability and the mammary glands lack TEB formation and ductal outgrowth, mammary development was analyzed in mice carrying an *igf1* midi (*Igf1^m*) allele (19). *Igf1^m* mice carry an insertional mutation in the *igf1* gene resulting in IGF-I levels that are 30% of wild-type levels and a reduction in body size that is intermediate between wild-type and *igf1* $-/-$ mice (20). In contrast to the *igf1* $-/-$ mice, the *igf1^m* mice are viable and fertile (20). Pubertal mammary glands of the *igf1^m* mice demonstrate reduced ductal branching (19). In contrast, analysis of the LID mice with a 75% reduction in circulating IGF-I levels demonstrated no deficits in mammary gland ductal growth, branching or lactation (19). Since circulating IGF-II is low in rodents postnatally, these data support the hypothesis that locally expressed IGFs are important for pubertal ductal branching. However, the exact roles of circulating and local IGF-I in TEB formation and ductal outgrowth are unclear. Moreover, mammary development has not been analyzed in the LID/ALSKO mice where circulating IGF-I is reduced to 10% of wild type levels. It is, however, worth noting that an important difference between humans and rodents in considering circulating IGF actions is that levels of IGF-II in human serum remain elevated postnatally. Thus, there is the potential that circulating levels of both IGF ligands have a role in normal breast development in humans.

Local IGF ligand expression and regulation in mammary development

Data from several laboratories have provided evidence that mRNAs for the IGFs, IGF-IR and the IGF binding proteins (IGFBPs) are all endogenously expressed in the murine mammary gland during postnatal mammary gland development (10,21–24). The two ligands, IGF-I and IGF-II, have distinct patterns of mRNA expression in mammary gland growth during puberty and pregnancy (10,24).

IGF-I

IGF-I is expressed in mammary stroma throughout postnatal development (10,25,26). Interestingly, IGF-I expression is also detectable in TEBs during pubertal ductal growth but mRNA expression becomes undetectable in the epithelium during post-pubertal and early pregnancy-induced growth. At mid-pregnancy, IGF-I mRNA expression is restored in the ductal epithelium, and by late pregnancy, IGF-I is expressed throughout the ductal and alveolar epithelium. While early studies from Ruan and Kleinberg indicated that stromal IGF-I is GH regulated, it is unclear whether epithelial IGF-I expression either in TEBs or in alveoli during late pregnancy is induced by GH or is distinctly regulated from stromal IGF-I expression.

IGF-II

IGF-II is co-expressed with IGF-I in TEBs during pubertal growth (25). Unlike IGF-I, IGF-II is expressed in the ductal epithelium during post-pubertal and early pregnancy-induced growth. By post-pubertal ages, IGF-II expression becomes non-uniformly distributed in ductal epithelium, a pattern that is maintained in ducts and alveoli during pregnancy (25). This nonuniform patterning of IGF-II expression is similar to the expression patterns of the progesterone receptor (PR) and prolactin receptor (PRLR) (27–32). Recent studies demonstrated that the non-uniform pattern of PR, PRLR and IGF-II requires the CCAAT/enhancer binding protein- β (C/EBP- β) transcription factor. Seagroves and colleagues showed that genetic deletion of C/EBP- β results in uniform expression of the PR and disruption of alveolar development (28,29). Further studies demonstrated that expression of the PRLR and IGF-II are similarly disrupted in the CEBP- β KO glands (27). Moreover, treatment of the CEBP- β KO mice with estrogen and progesterone (E/P) failed to re-establish non-uniform cellular expression in the KO glands, while in the wild type glands, E/P treatment enhanced patterning, including that of IGF-II. Two subsequent reports provided evidence that IGF-II expression is downstream of the PRLR in mammary epithelial cells (MECs) (33,34). Thus, while local IGF-I expression, like circulating IGF-I, is predominantly regulated by GH, local expression of IGF-II appears predominantly regulated by prolactin. These recent findings provide support for the hypothesis that IGF-I and IGF-II have distinct functions in promoting mammary development.

IGF ligand function in postnatal mammary development

Investigation into the function of IGF system components using transgenic animal models has provided insight into the individual roles of the IGF ligands and the IGF-1R during mammary gland growth, development and carcinogenesis. Initial studies on *Igf1*^{-/-} glands were reviewed previously (35). We focus here on how these and subsequent investigations provide support for the hypotheses that epithelial and stromal IGFs have distinct functions and, furthermore, that IGF-I and IGF-II may have overlapping as well as distinct functions during mammary development.

Epithelial vs. stromal actions of IGF-I in mammary development

The contributions of epithelial and stromal compartments in mediating mammary development have been the focus of numerous studies and also of reviews in this journal. These studies have revealed differential expression and/or function of growth factors, hormones and/or receptors in the epithelial and stromal compartments. The question of epithelial and stromal-specific functions of IGFs became of interest following the demonstration that IGF-I and IGF-II mRNAs were detected in epithelial cells in addition to stromal cells. As discussed above, glands from mice carrying a global deletion of *Igf1* show severe deficits in TEB formation and ductal outgrowth (35,36). Moreover, at least some part of ductal growth, specifically ductal branching, is dependent on local IGF-I (19). To test the function of epithelial IGF-I, we established mice with mammary epithelial-specific deletion of the *igf1* gene using Cre-loxP technology (37).

By expressing Cre-recombinase from the MMTV promoter, we deleted epithelial IGF-I during pubertal ductal growth when it is expressed in the TEBs. Moreover, since mice with a global *igf1* deletion have limited postnatal viability and systemic growth defects, we analyzed glands from mice heterozygous for IGF-I expression to determine the role of stromal IGF-I. These mice have a 50% reduction of IGF-I throughout both the stromal and parenchymal compartments of the mammary gland and, it should be noted, in circulation. However, since no alterations in mammary growth were observed with 75% reduction in circulating IGF-I (LID mice), we compared the phenotype of the heterozygous glands with those where only epithelial IGF-I was deleted to determine the role of stromal IGF-I.

The results of the analyses in virgin glands demonstrated that the glands from *igf-1*^{+/-} mice, showed no alterations in the total fat pad area or in ductal elongation. Similarly, a ≤50% loss of epithelial IGF-I had no effect on these parameters. In contrast, genotypes with greater than 50% reduction in epithelial IGF-I showed decreased branching complexity (37). In addition to morphometric analyses, we also analyzed expression of cyclins as a measure of cell cycle progression in the mice with epithelial-specific disruption or overall reduction in IGF-I expression (37). There were no significant changes in either D-type cyclin expression or DNA synthesis between genotypes. In contrast, cyclin A2 and B1 mRNA levels were decreased in mice heterozygous for the *igf1* null allele. Epithelial deletion of IGF-I had no effect on cyclin expression in these analyses. These results are consistent with observations from whole organ culture experiments on pubertal stage glands showing that exogenous IGF-I significantly induced expression of cyclins A2 and B1, important for S and G₂ progression, respectively (38). Interestingly, a previous report analyzing uterine epithelial proliferation in the absence of IGF-I also demonstrated an essential function in G₂, but not G₁, progression as well as decreased cell number (39). Using the ex vivo culture system, we further demonstrated that IGF-I is synergistic with epidermal growth factor (EGF)-related ligands in promoting DNA synthesis, and is essential for the EGF-related ligands to promote S-phase entry in MECs in the intact gland (38,40).

The *igf-1*^{+/-} mice also were used for analysis of stromal IGF-I function during stages when there is little detectable IGF-I expression in the epithelium (early- to mid-pregnancy) (37). Analysis of glands at day five of pregnancy (p.5.5) revealed reduced alveolar budding in the *igf-1*^{+/-} glands compared to wild type glands. Analysis of BrdU incorporation at this stage showed no significant difference between the two genotypes. At p14.5 the *igf-1*^{+/-} glands still showed reduced alveolar density, although the difference between the heterozygous and wild-type glands was less dramatic than at p5.5. Interestingly, by this time, quantification of the number of nuclei revealed more nuclei per alveolus, an increase in the number of Ki67-positive cells and an increase in cyclin D1 mRNA and protein in the *igf-1*^{+/-} glands compared to wild-type glands. These changes were accompanied by a reduction in milk protein expression at p14.5. These latter changes were reflected only at the protein level; levels of beta-casein mRNA and phosphorylated stat5 protein were unchanged in the *igf-1*^{+/-} glands at p14.5.

Based on these data, we concluded that IGF-I has distinct functions when expressed in stromal and epithelial locations. Specifically, IGF-I expressed in the TEBs during pubertal growth is necessary for normal ductal branching. In contrast, stromally produced IGF-I regulates S and G₂ cyclin expression. Consistent with our conclusions that paracrine actions of IGF-I promote epithelial proliferation, de Ostrovich et al recently showed that ectopic expression of IGF-I in myoepithelial cells enhanced epithelial proliferation in pubertal ducts (41). Since a previous study demonstrated complete lack of TEB formation and ductal outgrowth in IGF-I null glands, we expect that stromal and/or circulating IGF-I is essential for pubertal outgrowth and that 50% of wild type IGF-I levels is sufficient for normal ductal extension. It is interesting that a recent report showed an essential role for amphiregulin in ductal outgrowth and proliferation (42). The coordination of IGF-I and EGF ligand signaling in epithelial proliferation in our ex

vivo studies and the similar phenotype from loss of amphiregulin or IGF-I in TEB expansion and ductal outgrowth suggest that these two factors may work coordinately in pubertal ductal growth.

IGF-I is essential for initial alveolar development during pregnancy, and decreased IGF-I leads to hyperplasia in the epithelium by mid-pregnancy. We surmise that the hyperplastic phenotype represents “compensatory” proliferation. Similar delayed growth followed by compensatory hyperplasia has been described following loss of IGF-I in muscle using a Cre/loxP approach (43).

Finally, we recently demonstrated that several members of the family of IGFBPs, which function to regulate and localize the IGFs, are differentially expressed in the epithelial and stromal compartments and are particularly enriched in the stroma around the growing ductal structures (21). Thus, the IGFBPs are positioned to partition the IGFs and regulate their actions in the epithelial and stromal compartments. Taken together, these data support the hypothesis that temporal and spatial restriction of IGF-I and IGF-II expression during mammary development confers ligand-specific functions.

Evidence that IGF-I and IGF-II have distinct functions in mammary development

A major issue in unraveling functions of IGF-I and IGF-II is the potential for redundant or compensatory actions when expression of one ligand is disrupted. Thus, the above studies reveal only essential functions for IGF-I for which IGF-II is unable to compensate. In some cases, individual ligand function is clearly significant such as in the *igf-1*^{-/-} glands, which fail to form TEBs and to undergo ductal development even with systemic replacement of ovarian hormones. While local IGF-II expression is unable to compensate for global loss of IGF-I in initiating pubertal mammary epithelial growth, many questions remain. For example, it is unclear whether the expression of both IGF-I and IGF-II in TEBs represents redundant or distinct functions during ductal elongation and whether a double KO of epithelial IGF-I/IGF-II would result in a more dramatic phenotype than was seen with loss of epithelial IGF-I. Similarly, it is unknown whether the two ligands have distinct functions in stroma during phases of epithelial growth. Finally, both IGF-I and IGF-II are expressed in epithelium by late pregnancy; however, their pattern of expression is distinct at this time (uniform in the case of IGF-I and non-uniform in the case of IGF-II).

Further evidence that IGF-II has distinct functions is supported by subsequent studies with *igf2*^{-/-} mammary epithelium demonstrating that IGF-II is an essential component of prolactin mediated alveolar proliferation (33,34). The IGF-II null mice are 40% smaller than their littermates at birth and retain this dwarf phenotype proportionally into adulthood (44). Studies demonstrating local IGF-II function in the mammary gland were performed by Briskin et al. where IGF-II null epithelium was transplanted into cleared fat pads of wild-type mice resulting in a reduction in alveolar differentiation at mid-pregnancy (33). Since circulating and local IGF-I were normal in these transplants, it can be concluded that epithelial IGF-II has a unique role in normal alveolar development.

IGF Signaling receptors and expression during mammary development

Some of the questions about distinct functions for IGF-I and IGF-II may be answered by addressing functions of IGF signaling receptors in the mammary gland. For a recent review of insulin and IGF receptor structure and signaling pathways see Werner et al, 2008 (45). There are three types of potential IGF signaling receptors, IGF-IR, insulin receptor (IR), and hybridIR (see also reviews by Morehead and Belfiore in this issue). All of the IGF signaling receptor subtypes are heterotetramers formed in the endoplasmic reticulum containing two extracellular subunits and two transmembrane β subunits linked by disulfide bonds.

IGF-IR mRNAs are uniformly expressed throughout mammary epithelium at all stages of postnatal mammary gland development (10,23,25). Additionally, IGF-IR mRNA is detected in the mammary stroma during pubertal, post-pubertal and pregnancy-induced growth although at lower levels than in mammary epithelium (10,25). In contrast to the IGF-IR, there is less known about expression levels of the IR isoforms in MECs during development. However, the presence of both isoforms in breast cancer cell lines and in normal mouse and human MECs suggests that they may be important for normal mammary epithelial development.

The key to differences in IGF ligand actions in the mammary gland may be found in the differential ligand-receptor affinity (summarized in Table 1). Insulin has equal affinity for either IR isoform and binds the IGF-IR only at superphysiological concentrations (7). Likewise, the IGF ligands have high affinity for the IGF-IR and low affinity for IR-B (6,7). In contrast, IGF-II can stimulate IR-A and IGF-IR autophosphorylation with similar affinity (5) and binds both IR-A and the IGF-IR with affinity comparable to that of insulin and IGF-I for their cognate receptors (7). The ability of IGF-II to activate the IR-A isoform raises the possibility that this receptor might mediate IGF-II-specific actions in mammary development consistent with its proposed role via IGF-II in breast cancers (see Belfiore review in this issue). Finally, IGF-II also has high affinity binding to the IGF-IIR (Table 1), which acts to limit IGF-II availability but is not thought to promote cellular signaling (46–48).

The existence of hybridRs consisting of one IR α - β subunit and one IGF-IR α - β subunit has been verified in a number of mammalian tissues and cell types including breast cancer cells (6,7,49–54). HybridR formation is thought to occur via a stochastic method based on relative expression levels of each receptor (49,55). Characterization of hybridRs demonstrated that they have high affinity for IGF ligands and lower affinity for insulin (52,53,56). Further hybridR studies in rodent cells that lack endogenous receptors but over-express human receptor constructs have been used to address the differences in IR isoform ligand-binding properties (6–8). These reports contain conflicting data, particularly with regard to the ligand-binding properties of hybridRs containing IR-B. However, the recent reports by Slaaby and colleagues and Benyoucef et al, each demonstrated by three different methods that hybridRs containing IR-A or IR-B have approximately equal affinity for each ligand (6,8) (Table 1). Expression of hybridR in MECs has been shown in breast cancer cell lines (56). We also have detected the presence of hybridRs in mouse MECs and in protein extracts isolated from lactating glands (A.M. Rowzee, M.A. Stull and T.L. Wood, unpublished data). The affinity of the IGF ligands for the hybridR suggests a mechanism either to enhance IGF signaling or to inhibit insulin signaling in MECs. The possible functions of the hybridR as well as whether downstream signaling from the hybridR is similar or distinct from either the IGF-IR and IR remain undefined.

IGF-IR and IR function in mammary development

The importance of IGF-IR signaling in the mammary gland has been demonstrated in a variety of transgenic mouse models. Although a null mutation of the IGF-IR results in perinatal lethality in mice (12), rescue of IGF-IR null epithelium and transplantation into cleared mammary fat pads demonstrated that the IGF-IR is necessary for normal ductal outgrowth and proliferation of TEBs (57). Constitutive activation or overexpression of the IGF-IR in the mammary epithelium disrupts pubertal development and leads to hyperplasias and tumor formation (58,59) (reviewed by Morehead in this issue). Thus, the IGF-IR is necessary for normal development and potentially disrupts epithelial growth when overactivated.

Similar to the experiments for the IGF-IR null mammary epithelium, rescue experiments were performed with IR null epithelial cells transplanted into wild-type fat pads (60). These studies demonstrated that loss of the IR in MECs resulted in smaller alveoli during pregnancy-induced

growth indicating that the IR is also essential for normal growth and differentiation of MECs, specifically during alveolar differentiation.

A major issue surrounding systemic deletion of genes is the potential for compensation by other genes when the animal develops around the absence of a gene. More recently, a Cre-loxP approach has been used to delete the IR or IGF-IR in a tissue specific manner (61,62) thereby eliminating problems with embryonic/perinatal lethality as well as systemic effects of gene deletion. This approach will be important for temporal deletion of the IGF-IR and/or IR using mammary epithelial-specific promoters to determine additional roles for these receptors in mammary development at different stages in the absence of long-term compensatory changes.

Local IGF ligand and receptor expression in normal human breast

While several reports have focused on the correlation of circulating IGF-I and breast cancer risk, other studies have provided evidence for a role for locally expressed IGFs in both normal and malignant breast tissue. Results from these studies suggest that IGF-I is expressed locally in breast tissue primarily in stromal fibroblasts (63–65). In contrast to IGF-I, IGF-II is low or undetectable in normal breast tissue, in either epithelial or stromal cells but is expressed in fibroblasts associated with breast tumors (64–66) and in malignant epithelial cells (65). The IGF-IR has been localized predominantly to human breast epithelium by immunohistochemistry with weaker staining observed in endothelial cells (67). Similar studies of human breast tissue in pregnancy and lactation are not available nor are studies investigating expression of the IR or isoforms in normal breast epithelium. Both the IGF-IR and IR are expressed in malignant epithelial cells of certain breast cancers as reviewed extensively in other articles in this journal. Zhang and colleagues included the non-transformed human breast epithelial cell line, MCF-10A, in their analysis of IR isoform and IGF-IR expression and showed that all receptors were expressed in the 10A cells but the ratio of IR-A to IR-B was less than what was observed in breast cancer tissues or cell lines (68).

The localization of IGF-I in the stromal compartment and the prominent expression of the IGF-IR in the epithelium is similar in the rodent mammary gland and human breast. This suggests an essential role for locally-produced IGFs in stromal-epithelial interactions and control of epithelial growth in the human breast. It is unknown whether IGF-I or IGF-II are present in human breast epithelial cells at specific times such as in the proliferating terminal ductal lobular units or in the alveoli during late pregnancy similar to their expression in developing mouse glands.

Concluding remarks and future questions

Model for IGF ligand/receptor regulation of mammary development

Based on functions of IGF-I, IGF-II, IGF-IR and IR demonstrated thus far, it is tempting to begin building a model for coordinated IGF ligand/receptor function in development of specific stages. Such a model can be based on the previous genetic studies elucidating distinct and overlapping functions for the IGFs through the IGF-IR and IR in fetal development. Individual deletion of IGF-I or IGF-II in mice each results in reduction in fetal growth, however, the combined deletion of the two ligands causes an even greater reduction in fetal growth (11,12, 44). Moreover, the extent of fetal growth retardation in the IGF-I/IGF-II double mutants is similar to that seen in the double IGF-II/IGF-IR mutants but greater than the phenotype of IGF-I/IGF-IR double mutants (11,12).

Similarly, genetic experiments of global versus conditional IGF ligand deletion have demonstrated the potential for unique and overlapping functions for IGF-I and IGF-II. It is clear that IGF-I has a unique function in initial TEB formation. Since local IGF-II does not

compensate for loss of IGF-I, it is possible that circulating IGF-I contributes to TEB formation. Once initiated, IGF-I and IGF-II may have overlapping functions in promoting ductal outgrowth possibly through both IGF-IR and IR. Based on this model, we predict that double KO's of the IGF-IR and IR or of the IGF-IR and IGF-II in epithelium will cause a greater phenotype than was observed from loss of epithelial IGF-IR alone. However, indirect effects of the IGFs acting on stromal receptors cannot be excluded for a role in mediating epithelial growth. Such indirect effects require the ability to inactivate the receptors in the mammary stromal compartment without compromising overall viability of the animal.

Extending a model to pregnancy stages, the evidence suggest a unique role for stromal IGF-I through the IGF-IR in early alveolar budding while IGF-II has a distinct role in alveolar proliferation at mid-pregnancy. It will be of interest for further investigations to determine IR and IR isoform expression and function during pregnancy stages.

IGFs as potential regulators of mammary epithelial stem/progenitor cells

Given the importance of IGF signaling in mammary development, as well as the early timing of expression of both IGF ligands and receptors within the epithelium, it is likely that IGF signaling contributes to both mammary epithelial stem cell maintenance and renewal as well as progenitor cell expansion. The uniform expression of IGF-IR throughout the developing mammary epithelium from early growth stages supports the idea that it is expressed in immature as well as differentiated cell populations (10,24). Moreover, both IGF ligands are expressed in mammary stroma at these early stages, and IGF-II is expressed throughout the mammary epithelium during puberty (10,24,69). As outlined above, it is likely that both IGF-I and IGF-II stimulate the IGF-IR during early growth as has been shown for IGF ligand actions in fetal growth. At these early stages, a role for the IR-A is likely as well since its expression is characteristic of immature cells. It is possible then that IR-A mediates IGF-II actions on immature cells.

IGF signaling impacts several developmental stages of the mammary gland critical for lineage determination or expansion. The TEBs consist of an enriched population of epithelial progenitors, the cap cells and body cells. With the aid of surrounding stromal cells, growth, differentiation and luminal formation within the TEB drives outgrowth and branching of the early ductal structure. Loss of IGF-I prevents formation of TEBs (36), and IGF-IR deficiency in MECs compromises embryonic mammary bud development and proliferation of the TEB cap cells (57,70). Loss of epithelial IGF-I compromises ductal branching and 50% reduction of IGF-I in mammary stroma reduces early alveolar budding (37). Very little is known about the lineage specific requirements for IGF signaling in the developing gland. Transplantation of IGF-IR null epithelial cells demonstrated that the IGF-IR is required for proliferation of cap cells, a putative stem cell and myoepithelial precursor (57,71,72). In contrast, lack of IGF-IR had no effect on growth of the body cells or putative luminal progenitors of TEBs (57,71,72). This phenotype, therefore, suggests a myoepithelial cell defect due to cap cell deficiency. Myoepithelial cells dictate normal luminal polarization, and have been postulated to inhibit dysregulated luminal cell growth (73,74). IGF, therefore, could be important for myoepithelial cell survival as well as stem cell renewal during early development. Two isoforms of IRS, downstream targets of IGF signaling, are differentially expressed in the mammary gland epithelium, supporting two different IGF networks that may be lineage specific (75). These findings suggest that IGF signaling may contribute to self-renewal as well as lineage determination.

Accumulating data in other cell types, including human embryonic, neural, and hematopoietic stem cells, support the hypothesis that IGFs promote stem cell renewal (76–79). Recent studies of human embryonic stem cell renewal have demonstrated that signaling of IGF-IR in these cells with stromal-derived IGF-II supports their self renewal (77,78). In agreement with a role

for IGFs in stem cell renewal, microarray studies of human stem-derived mammosphere cultures, showed that IGF signaling is up-regulated in these stem- and progenitor-amplified cultures (80). Additionally, IGF-I signaling regulates cell fate determination of neural stem cells (76,81).

The molecular and cellular mechanisms of IGF signaling in each of the developmental stages of the mammary gland are clearly complex. The use of different ligand-receptor combinations combined with additional growth factor and hormone signals dictates a highly organized orchestration of growth, survival and repair. It will be interesting for future studies to uncover the lineage specific responses of the mammary gland to IGF signaling, and to better understand this cellular signaling network. Likely, these efforts will contribute greatly to understanding the mechanisms of initiation and progression in breast cancers where dysregulated IGF signaling plays a major role.

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Abbreviations

IGF	Insulin like-growth factor
IGF-IR	IGF-type I receptor
IR	insulin receptor
hybridR	IGF-IR:IR hybrid receptor
GH	growth hormone
LID	liver-specific IGF-I deletion
ALS	acid labile subunit
IGFBP	IGF binding proteins
TEB	terminal end bud
PR	progesterone receptor
PRLR	prolactin receptor
C/EBP-β	CCAAT/enhancer binding protein-beta

E/P	estrogen/progesterone
KO	knockout
MEC	mammary epithelial cell
MMTV	mouse mammary tumor virus
EGF	epidermal growth factor
BrdU	bromodeoxyuridine

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Table 1
Relative Affinities of Ligands for IGF System Receptors

	IC50 nM			
	Insulin	IGF-I	IGF-II	Reference
IGF-IR	>30	0.2	0.6	(7)
	3.8	0.019		(8)
	>1 μ M	0.5		(6)
IR-A	0.9	41		(82)
	0.2	>30	0.9	(7)
	0.3	9	2.2	(6)
IR-B	1.6	390		(82)
	0.3	>30	11	(7)
	0.5	90	10	(6)
Hybrid-A	3.7	0.3	0.6	(7)
	2.6	0.017	0.18	(8)
	70	0.5	0.7	(6)
Hybrid-B	>100	2.5	15	(7)
	2.8	0.012	0.19	(8)
	76	0.3	0.3	(6)
IGF2R	Undetectable	0.4 μ M	0.2–1.0	(83,84)