Development of Rudimentary Structure of Mammary Gland

Subjects: Biochemistry & Molecular Biology

Contributor: Indrajit Chowdhury , , CY Kuo

The mammary gland is a compound, branched tubuloalveolar structure and a major characteristic of mammals. The mammary gland has evolved from epidermal apocrine glands, the skin glands as an accessory reproductive organ to support postnatal survival of offspring by producing milk as a source of nutrition. The mammary gland development begins during embryogenesis as a rudimentary structure that grows into an elementary branched ductal tree and is embedded in one end of a larger mammary fat pad at birth. At the onset of ovarian function at puberty, the rudimentary ductal system undergoes dramatic morphogenetic change with ductal elongation and branching. During pregnancy, the alveolar differentiation and tertiary branching are completed, and during lactation, the mature milk-producing glands eventually develop. The early stages of mammary development are hormonal independent, whereas during puberty and pregnancy, mammary gland development is hormonal dependent. The mammary gland develops as a rudimentary structure from a thickening under the ventral skin during embryogenesis. This rudimentary structure grows into a rudimentary branched ductal tree embedded in one end of a larger mammary fat pad at birth. The embryonic development of the mammary gland is a series of several hormone-independent specialized events.

mammary gland signaling development

1. Introduction

The mammary gland is a compound, branched tubuloalveolar structure, and a major characteristic of mammals. The mammary gland evolved from the epidermal apocrine gland, the skin glands as a bilateral accessory reproductive organ located on the ventral surface of the body [1]. Mammary glands produce milk as a source of nutrition for supporting the postnatal survival of offspring for reproductive success in all mammals [2]. Morphologically, mammary glands are formed by several different types of cells. The epithelial cells elaborate the ductal network of the gland and maximize the surface area within a constrained volume, whereas a variety of stromal cells or connective tissues with extracellular matrix (ECM) protein supports the mammary glands. The major components of stromal connective tissues are adipocytes, which constitute the mammary fat pad and retain the embedded ductal network; fibroblasts which support the hematopoietic system; vascular endothelial cells which support the blood vessels; a variety of innate immune cells (both macrophages and mast cells); and nerves [3][4]. The fatty stroma is the supportive network for the epithelium bi-layered structure and provides nutrients, blood supply, and immune defenses besides the physical structure to the gland. Importantly, each specific stromal cell secretes instructive signals for specific aspects of the development and function of the epithelium [5]. There are two

main types of epithelium in the mammary gland, namely, luminal and basal. The luminal epithelium forms the inner layer of the ducts as a laticiferous duct. It surrounds the hollow lumen that differentiates into the milk-producing secretory alveoli or lobules. In contrast, the basal epithelium consists of myoepithelial cells that form the outer layer of mature mammary ducts. It also harbors stem and progenitor cells, which form both luminal and myoepithelial cells/layer [6]. The epithelium ensheathes by one of the main types of ECM, the basement membrane (BM), which separates the epithelium from the stroma and influences the development of the mammary gland [7]. Thus, BMs surround three cell types in the mammary gland, namely, the epithelium, the endothelium of the vasculature, and the adipocytes. In males, mammary glands are present as a rudimentary structure and generally nonfunctional form.

2. Human Rudimentary Structure of the Mammary Gland

The human mammary gland development is initiated during embryonic life from the parenchyma as a single epithelial ectodermal bud. The first visible indication of primitive mammary bud development can be recognized on day 35 (week 5), with the proliferation of paired areas in the epidermis of the thoracic region [8]. Subsequently, two distinct mammary ridges or milk streaks are formed between the fetal axilla and inguinal region. By the end of week six, the mammary ridges become regressed to two areas in the thoracic region (2nd–6th rib). Two solid epithelial masses as mammary bud begin to grow downward into the underlying mesenchyme [8]. The solid epithelial masses evaginate into the underlying mesenchyme and are surrounded by fibroblast-like cells within a dense collagenous stroma. During the seventh and eighth weeks of gestation, the mammary parenchyma invades the stroma, which appears as a raised portion called the mammary disc.

On week 9, the mammary placodes grow inward further as a cone stage. Between the 10th and 12th week, epithelial buds sprout from the invading placodes and transform into globular shapes with the notching of the epithelial–stromal border as a nascent stage. Parenchymal branching occurs during the 13th through 20th weeks. Between the 12th and 16th weeks of gestation, the nipple and areola form in the epidermis and overlie the developing glands with the differentiation of the mesenchymal cells into fibroblasts, smooth muscle cells, capillary endothelial cells, and adipocytes. On week 15 occurs branching of 15–25 solid epithelial cords, called the branching stage. On week 20, the solid mammary cords canalize; the epidermis in the region of the nipple becomes depressed and forms the mammary pit [8]. After that, the cuboidal epithelial cell line forms a bilayer around the ducts. The luminal layer rapidly acquires the characteristics of secretory cells, whereas the basal layer becomes myoepithelial. By six months of gestation, the basic tubular architecture of the fetal glands becomes established. Branching continues, and canalization of the cords occurs, forming the primary milk ducts by 32 weeks gestation [1]. At 32 weeks' gestation, the ducts open onto the area, which develops into the nipple [9]. The adipose tissue of the mammary gland develops from connective tissue that has lost its capacity to form fibers and support further growth of parenchyma [10]. The fat islands are within a fibrous connective stroma that separates the ducts. The primitive secretory epithelial cells, which become functional near the end of gestation, respond to the lactogenic hormones of pregnancy [8]. In humans, mammary glands develop similarly in female and male fetuses

[11]. Due to difficulties in precisely establishing the day of conception, the phases of mammary gland development are correlated with embryonal or fetal size [8].

The newborn mammary glands are a very primitive structure, composed of ducts ending in short ductules or epidermal ridges (milk lines) lined by one to two layers of epithelial and one layer of myoepithelial cells. The epithelial cells have eosinophilic cytoplasm and lipid droplets with typical apocrine secretion and fine cytoplasmic vacuolization. Therefore, colostrum can be expressed from the infant's mammary glands shortly after birth. This is attributed to the pro-lactation hormones present in the fetal circulation at birth. The secretory activity of the newborn glands subsides within 3–4 weeks [12]. The BM of the mammary bud and early projections contain type IV and VII collagen and laminin-a3, and the epithelium displays β1, β4, and α6 integrin expression [13]. However, BM signaling in the embryonic gland is unknown [13]. Regression of the mammary gland usually occurs by four weeks postpartum and coincides with a decrease in the secretion of prolactin from the anterior pituitary gland of the infant [10]. After birth, the mammary gland becomes quiescent until the onset of puberty. Thus, the ducts in the newborn breast are rudimentary and have small, club-like ends that regress soon after birth.

3. Mouse Rudimentary Structure of the Mammary Gland

The mouse model combined with tissue recombination techniques was used to generate chimeric glands to understand the morphogenesis and lineage commitment events during embryonic stages of mammary gland development. Moreover, the mouse model supports different stages of development at specific time points in a genetically identical group and supports conducting extensive in vivo studies. The milk or mammary lines formation initiation begins mid-gestation on an embryonic day (E) 10.5 [14]. Within 24–36 h of formation, the mammary line resolves into five pairs of lens-shaped placodes in the mouse. The epithelial placodes are a lens-shaped thickening of surface ectoderm formed by several layers of columnar cells invaginate from the ectoderm. These placodes invade the presumptive mammary mesenchyme to create a naive ductal network [15][16]. On E12.5, each placode expands and invaginates into the underlying mesenchyme to form a mammary bud [17][18]. The mammary epithelial cells proliferate downward and lead to bud growth as mammary sprouts into the dense mesenchyme until it reaches the developing mammary fat pad located within the dermis. The mammary fat pad consists of a loose collection of preadipocytes originating from mesenchymal condensation on E14. The onset of ductal branching, the morphogenesis starts on E16. The mammary fat pad is formed by the skin overlying the primary mammary mesenchyme and is remodeled into a second stromal compartment filled with preadipocytes [15]. At this stage, primary cord or mammary sprouts start dichotomous branching and give rise to the rudimentary ductal tree with a primary duct with 10–15 secondary branches present at birth. Concurrently, a ductal lumen is formed, and the skin overlying the primary mammary mesenchyme remodel into a typical nipple structure. This process involves thickening the epidermis, suppressing the hair follicle development, and invagination of a concentric ring of keratinocytes that forms the nipple sheath [19]. Thus, in female mice, the simple nascent structure is formed by iterative branching and maintains a continuous BM at the epithelial–mesenchymal interface. In contrast, in male mice, testosterone (T) elicits condensation of the mesenchyme around mammary buds and triggers the destruction of the epithelial rudiment by day 16 [20].

Upon completion of embryonic development, both rodents and humans have similar rudimentary mammary parenchymal structures or a superficial branch organotypic epithelial structure as mammary buds. Mammary buds are sphere structures of concentrically arrayed mammary epithelial cells hanging from the skin by a stalk of epidermal-like cells surrounded by condensed mammary mesenchyme [13][15][21].

4. Regulators of Embryonic Rudimentary Mammary Development

The embryonic or prenatal mammary development is regulated by multiple genes and transcriptional and translational products through complex signaling pathways based on mouse model studies and surrogate model systems (in vitro culture in 3D gels composed of extracellular matrix components). The initial branching morphogenesis of the embryonic mammary gland is a hormone-independent process [1][22]. Studies have demonstrated that mice have no apparent effects of the growth hormone receptor (GHR), estrogen receptor α/β (ERα/β), prolactin receptor (PRLR), and progesterone receptor (PR) during embryonic mammary development [1][22]. Based on mouse model and mammary cell line studies, wingless-related integration site (Wnt)/β-catenin, fibroblast growth factors (FGF), hedgehog (Hh), insulin-like growth factor-1 (IGF-1), parathyroid hormone-related protein (PTHrP), neuregulin 3 (NRG3), and their receptors are key signaling molecules during embryonic mammary development [15]. These signaling molecules regulate a wide range of transcription factors (TFs) from the Homeobox gene family (HOX), GATA binding protein 3 (GATA3), and the T-box family (TBX) in the endoderm or mesoderm.

Wnt/β-catenin signaling is universally required by all mammary placodes and ectodermal appendages [17]. There are nineteen Wnt ligands, and ten Frizzled receptors are known [23]. Wnt ligands act as morphogens that provide positional information to neighboring cells through canonical and non-canonical intracellular signaling [24][25]. In the canonical signaling pathway, the binding of Wnt with a Frizzled receptor and a low-density lipoprotein receptor-related protein 5 or 6 (LPR) causes the disassembly of a multiprotein complex containing glycogen synthase kinase 3 (GSK3), casein kinase 1α (CK1α), axin, and adenomatous polyposis coli (APC). This process supports the translocation of β-catenin into the nucleus and binds lymphoid enhancer-binding factor 1 (LEF1) transcription factor [26]. In the non-canonical signaling, the β-catenin-independent pathway is activated either by the change in cell polarity or cytoskeletal rearrangement through the Planar Cell Polarity (PCP) pathway or the intracellular Wnt/Ca^{2+} pathway [26]. The PCP pathway directs asymmetrical cytoskeletal rearrangement and cellular polarity through disheveled JNK and Rho family GTPase, whereas Wnt/Ca^{2+} pathways, Frizzled acts through G-proteins to activate phospholipase C (PLC), resulting in activation of various transcriptional factors [26]. A partial list of Wnt target genes is documented in mammary tissues, including *Cyclin D1, c-Myc, Wisp1, Wrch1, Stra6, Stromelysin-1, Cox-2*, and *Twist* mammary development [27]. Both loss- and gain-of-function experiments have demonstrated that canonical epithelial and mesenchymal Wnt/β-catenin signaling is critical for the initial mammary lines, placodes, and mature mammary bud development [17][28][29][30]. Disruption of Wnt signaling within the developing epidermis through transgenic expression of the secreted Wnt inhibitor dickkopf-related protein 1 (DKK1) abolish all morphologic evidence of mammary development. DKK1 suppresses early canonical Wnt signaling, subsequent

Wnt10b induction along the mammary line, and all placodal growth [17]. In contrast, activation of Wnt signaling results in the accelerated formation of enlarged mammary placodes [17][31]. Several other Wnt isoforms (Wnt3, Wnt6, and Wnt10b) are activators of early β-catenin signaling. Wnt isoforms express diffusely throughout the ectoderm along with the genetic hierarchy of factors, including ventral bone morphogenetic protein-4 (BMP4), dorsal T-box transcription factor-3 (TBX3), neuregulin-3 and somatic fibroblast growth factor-10 (*FGF10*), and act upstream to define the dorsal–ventral position along the mammary line [32][33]. The whole mount in situ hybridization studies suggested that Wnt10b (formerly Wnt12) is expressed in the mammary buds, E11/12 to E14/15 [34]. Therefore, Wnt10b regulates canonical Wnt/β-catenin signaling in mammary bud development and acts on epithelial or mesenchymal components [27]. As a downstream component from β-catenin and Lef1, Wnt signaling promotes the development of mammary rudiments by upregulation of the homeobox genes *Msx1* and *Msx2* [27]. The microarray studies have demonstrated that β-catenin target genes represent an essential module of the PTHrP-induced mammary mesenchyme specification process [35].

Similar to Wnt/β-catenin signaling, FGF signaling is essential to the early stages of mammary development and acts in parallel with Wnt signaling [15]. There are 23 FGF ligand members. However, only a few subsets have been studied in the mammary gland [36][37]. The inhibitor of Wnt signaling does not alter the expression of *FGF10* or FGF receptor 1 (*FGFR1*) [17][31]. *FGF10* is expressed in the developing mammary line, whereas *FGFR2β* is expressed within the developing mammary epithelial placodes. Interestingly, *FGF10* and *FGFR2β* genes knockout mice cannot form four pairs of placodes (number 1, 2, 3, and 5) [38][39]. FGF members signal in a paracrine manner from mesenchymal cells to epithelial cells. FGF binding with FGFR activates downstream signaling including the Ras-Raf-MEK-ERK and PI3K-Akt pathway, resulting in cell survival and proliferation.

Hedgehog (Hh) signaling pathway plays a crucial role in epithelial–mesenchymal interactions, cell differentiation, promoting proliferation, patterning, and survival during embryonic development [40]. Upon stimulation of the Hh pathway, the zinc finger transcription factors Gli2 and Gli3 are activated and promote transcription of the direct target gene Gli1 [41][42]. Knockdown and mutant studies have shown that somatic Gli3 regulates expression of *FGF10*, which in turn signals to ectodermal *FGFR2b* and thence, to Wnt10b [38][39]. Gli3 encodes a microtubule-bound transcription factor that phosphorylates to generate a repressor (CiA), or proteolytically cleave to generate a repressor (CiR), which regulates mammary bud formation [43]. Interestingly, GliA/GliR ratio (Gli activator forms to Gli repressor forms) provides a crucial developmental signal threshold for buds 3 and 5 in mice [4].

IGF-1 and insulin-like growth factor receptor (IGF1R) signaling support embryonic mammary bud morphogenesis through RhoGTPase activating (Rho-GAP) family and insulin receptor substrate-1/2 (IRS) effector protein in the epithelial–mesenchymal interactions [44]. IGF-1 is produced in the liver in response to the pituitary growth hormone (GH). Studies have shown that embryos deficient in P190-B, a member of the Rho-GAP family that interacts with integrins, had smaller mammary buds due to the defect in both compartments, mainly by lower proliferation of the epithelial bud and the aberrant underlying mesenchyme (45). A similar phenotype was observed in embryonic mammary buds lacking IRS-1/2 [45].

PTHrP and PTH1R signaling from epithelium to mesenchyme supports the formation of a rudimentary ductal tree, nipple, and nipple sheath and determines epithelial cell fate [4][46]. PTHrP is secreted by mammary epithelial cells and sensitize mammary mesenchymal cells [46], whereas PTH1R is expressed in the mesenchyme underlying the developing bud. The disruption of either the PTH1R or PTHrP gene in mice fails the rudimentary ductal tree and formation of the nipple [19][47]. An autocrine BMP4 acts as a downstream factor that triggers PTHrP signaling to support ductal outgrowth in the mammary mesenchyme [35][46]. Other studies have demonstrated that the treatment of mice in a combination of BMP4 and PTHrP enhance matrix metalloproteinase 2 (MMP2) activities in mesenchymal cells. In contrast, MMP inhibitors block PTHrP dependent mammary bud outgrowth in culture [35]. PTHrP signaling is acts to sensitize the primary mesenchyme to pre-existing BMP4 expression in the ventral dermis. This process induces the expression of a subset of specific genes such as *Msx2* transcription factor and MMP2 in the mesenchyme, which mediates the various morphogenetic tasks in initiating ductal morphogenesis from the bud [32]. Similarly, overexpression of PTHrP in basal keratinocyte converts dermis to mammary mesenchyme and suppresses hair follicle formation [19], and ultimately, supports mammary gland development. Ablation of epithelial SHH signals transforms mice hair follicles into a mammary-gland-like structure [48].

NRG3, a member of the epidermal growth factor (EGF) family, is critical for embryonic placode formation by augmenting or facilitating Wnt signaling [49]. NRG3 and its receptor ErbB4 are first expressed in the lateral plate mesoderm underlying the ectoderm where the mammary buds subsequently develop, immediately prior to the sequential development of each bud or placode, and promote mammary morphogenesis [49][50][51]. Once NRG3 binds to ErbB, ErbB dimerizes with another ErbB monomer or with one of three related receptors—ErbB2, ErbB3, or ErbB4, in order to exert its downstream effects to support normal embryonic placode formation [51][52]. Studies have shown that EGFs act as a mitogen for both epithelial and stromal cells [53].

5. Transcriptional Factors

Multiple transcription factors are involved in placode formation and mammary ductal outgrowth, including Gli. A T-box-containing transcription factor (T-box) is essential for placode formation. T-box3 (TBX3) is expressed in the mammary line and developing placodes. Lacking TBX3 in mice fails to produce mammary placodes 1, 3, 4, and 5 and fails to express the placodal markers Wnt10b and lymphoid enhancer-binding factor 1 (Lef-1) [54]. In mice, Lef1 is normally expressed in the epithelial cells of the mammary buds at E11/12 and subsequently induces the condensation of mesenchymes that surround each bud by E14/15 [19]. Induction of Lef1 expression depends on paracrine signaling from the mammary epithelium to the mesenchyme mediated by PTHrP and PTHR1 [19]. In humans, Tbx3 haploinsufficiency is associated with the ulnar-mammary syndrome and severe mammary hypoplasia, and sometimes complete loss of mammary glands [55]. TBX3 expression within the mammary line depends on both FGF and Wnt signaling. TBX3 expression up-regulates the expression of Wnt and FGF signaling pathways for complete mammary line development and transition to placode formation. Thus, TBX3 is downstream and upstream of Wnt and FGF signaling as a paradigm for T-box [15]. The orientation of ectodermal cells, mammary line specification, and placode formation is regulated conversely by bone morphogenic protein 4 (BMP4), which negatively regulates TBX3. Other homeodomain-containing transcription factors, including *Msx1* and *Msx2*, are

expressed differentially in the epithelium. Interestingly, *Msx2* is expressed only in mammary buds' mesenchyme [46][56][57]. Interestingly, the loss of either *Msx1* or *Msx2* alone does not affect the formation of the mammary buds, whereas loss of *Msx2* affects nipple formation and bud outgrowth [46][57]. Another homeodomain-containing transcription factor, Hoxc6, along with *Msx2* are required for mammary ductal outgrowth, since, just before ductal sprouting, *Msx2* is expressed in the mammary mesenchyme due to PTHrP/BMP4 signaling [46]. Ultimately, Hox gene regulates epithelial mammary bud regulatory elements (MBRE). However, the complete signaling network during embryonic mammary development is incompletely understood.

These complex signaling networks regulate the epithelial-to-mesenchymal transition (EMT) and support the development of the mammary gland. In addition, epithelial cells lose polarity and adhesion to become mesenchymal cells with migration and invasion properties.

References

1. Hovey, R.C.; Trott, J.F.; Vonderhaar, B.K. Establishing a framework for the functional mammary gland: From endocrinology to morphology. J. Mammary Gland Biol. Neoplasia 2002, 7, 17–38.
2. Peaker, M. The mammary gland in mammalian evolution: A brief commentary on some of the concepts. J. Mammary Gland Biol. Neoplasia 2002, 7, 347.
3. Muschler, J.; Streuli, C.H. Cell-matrix interactions in mammary gland development and breast cancer. Cold Spring Harb. Perspect. Biol. 2010, 2, a003202.
4. Watson, C.J.; Khaled, W.T. Mammary development in the embryo and adult: A journey of morphogenesis and commitment. Development 2008, 135, 995–1003.
5. Sternlicht, M.D. Key stages in mammary gland development: The cues that regulate ductal branching morphogenesis. Breast Cancer Res. 2006, 8, 201.
6. Visvader, J.E. Keeping a breast of the mammary epithelial hierarchy and breast tumorigenesis. Genes Dev. 2009, 23, 2563–2577.
7. Streuli, C.H. Cell adhesion in mammary gland biology and neoplasia. J. Mammary Gland Biol. Neoplasia 2003, 8, 375–381.
8. Parmar, H.; Cunha, G.R. Epithelial-stromal interactions in the mouse and human mammary gland in vivo. Endocr.-Relat. Cancer 2004, 11, 437.
9. Javed, A.; Lteif, A. Development of the Human Breast. Semin. Plast. Surg. 2013, 27, 5–12.
10. Vorherr, H. The Breast: Morphology, Physiology and Lactation; Academic Press: London, UK, 1974.

11. Howard, B.A.; Gusterson, B.A. Human breast development. J. Mammary Gland Biol. Neoplasia 2000, 5, 119.
12. Russo, J.; Russo, I.H. The Mammary Gland: Development, Regulation and Function; Neville, M.C., Daniel, C.W., Eds.; Plenum Publishing Corporation: New York, NY, USA, 1987; pp. 67–93.
13. Jolicoeur, F. Intrauterine breast development and the mammary myoepithelial lineage. J. Mammary Gland Biol. Neoplasia 2005, 10, 199.
14. Sakakura, T. Mammary embryogenesis. In The Mammary Gland: Development, Regulation and Function; Neville, M.C., Daniel, C.W., Eds.; Plenum Publishing Corporation: New York, NY, USA, 1987; pp. 37–66.
15. Hens, J.R.; Wysolmerski, J.J. Key stages of mammary gland development: Molecular mechanisms involved in the formation of the embryonic mammary gland. Breast Cancer Res. 2005, 7, 220.
16. Hinck, L.; Silberstein, G.B. Key stages in mammary gland development: The mammary end bud as a motile organ. Breast Cancer Res. 2005, 7, 245.
17. Chu, E.Y.; Hens, J.; Andl, T.; Kairo, A.; Yamaguchi, T.P.; Brisken, C.; Glick, A.; Wysolmerski, J.J.; Millar, S.E. Canonical WNT signaling promotes mammary placode development and is essential for initiation of mammary gland morphogenesis. Development 2004, 131, 4819.
18. Veltmaat, J.M.; Van Veelen, W.; Thiery, J.P.; Bellusci, S. Identification of the mammary line in mouse by Wnt10b expression. Dev. Dyn. 2004, 229, 349.
19. Foley, J.; Dann, P.; Hong, J.; Cosgrove, J.; Dreyer, B.; Rimm, D.; Dunbar, M.; Philbrick, W.; Wysolmerski, J. Parathyroid hormone-related protein maintains mammary epithelial fate and triggers nipple skin differentiation during embryonic breast development. Development 2001, 128, 513.
20. Kratochwil, K. Tissue combination and organ culture studies in the development of the embryonic mammary gland. In Developmental Biology: A Comprehensive Synthesis; Gwatkin, R.B.L., Ed.; Plenum Press: New York, NY, USA, 1987; pp. 315–334.
21. Masso-Welch, P.A.; Darcy, K.M.; Stangle-Castor, N.C.; Ip, M.M. A developmental atlas of rat mammary gland histology. J. Mammary Gland Biol. Neoplasia 2000, 5, 165.
22. Hennighausen, L.; Robinson, G.W. Signaling pathways in mammary gland development. Dev. Cell 2001, 1, 467.
23. Staal, F.J.; Chhatta, A.; Mikkers, H. Caught in a Wnt storm: Complexities of Wnt signalling in hematopoiesis. Exp. Hematol. 2016, 44, 451–457.
24. McNeill, H.; Woodgett, J.R. When pathways collide: Collaboration and connivance among signalling proteins in development. Nat. Rev. Mol. Cell Biol. 2010, 11, 404.

25. van Amerongen, R.; Nusse, R. Towards an integrated view of Wnt signaling in development. Development 2009, 136, 3205.
26. Komiya, Y.; Habas, R. Wnt signal transduction pathways. Organogenesis 2008, 4, 68–75.
27. Brennan, K.R.; Brown, A.M. Wnt proteins in mammary development and cancer. J. Mammary Gland Biol. Neoplasia 2004, 9, 119.
28. Bouras, T.; Pal, B.; Vaillant, F.; Harburg, G.; Asselin-Labat, M.L.; Oakes, S.R.; Lindeman, G.J.; Visvader, J.E. Notch signaling regulates mammary stem cell function and luminal cell-fate commitment. Cell Stem Cell 2008, 3, 429–441.
29. Lindvall, C.; Evans, N.C.; Zylstra, C.R.; Li, Y.; Alexander, C.M.; Williams, B.O. The Wnt signaling receptor Lrp5 is required for mammary ductal stem cell activity and Wnt1-induced tumorigenesis. J. Biol. Chem. 2006, 281, 35081–35087.
30. Lindvall, C.; Zylstra, C.R.; Evans, N.; West, R.A.; Dykema, K.; Furge, K.A.; Williams, B.O. The Wnt co-receptor Lrp6 is required for normal mouse mammary gland development. PLoS ONE 2009, 4, e5813.
31. Eblaghie, M.C.; Song, S.J.; Kim, J.Y.; Akita, K.; Tickle, C.; Jung, H.S. Interactions between FGF and Wnt signals and Tbx3 gene expression in mammary gland initiation in mouse embryos. J. Anat. 2004, 205, 1.
32. Cowin, P.; Wysolmerski, J. Molecular mechanisms guiding embryonic mammary gland development. Cold Spring Harb. Perspect. Biol. 2010, 2, a003251.
33. Incassati, A.; Chandramouli, A.; Eelkema, R.; Cowin, P. Key signaling nodes in mammary gland development and cancer: β-catenin. Breast Cancer Res. 2010, 12, 213.
34. Christiansen, J.H.; Dennis, C.L.; Wicking, C.A.; Monkley, S.J.; Wilkinson, D.G.; Wainwright, B.J. Murine Wnt-11 and Wnt-12 have temporally and spatially restricted expression patterns during embryonic development. Mech. Dev. 1995, 51, 341.
35. Hens, J.; Dann, P.; Hiremath, M.; Pan, T.C.; Chodosh, L.; Wysolmerski, J. Analysis of gene expression in PTHrP−/− mammary buds supports a role for BMP signaling and MMP2 in the initiation of ductal morphogenesis. Dev. Dyn. 2009, 238, 2713.
36. Hynes, N.E.; Watson, C.J. Mammary gland growth factors: Roles in normal development and in cancer. Cold Spring Harb. Perspect. Biol. 2010, 2, a003186.
37. Pond, A.C.; Bin, X.; Batts, T.; Roarty, K.; Hilsenbeck, S.; Rosen, J.M. Fibroblast growth factor receptor signaling is essential for normal mammary gland development and stem cell function. Stem Cells 2013, 31, 178–189.
38. Mailleux, A.A.; Spencer-Dene, B.; Dillon, C.; Ndiaye, D.; Savona-Baron, C.; Itoh, N.; Kato, S.; Dickson, C.; Thiery, J.P.; Bellusci, S. Role of FGF10/FGFR2b signaling during mammary gland

development in the mouse embryo. Development 2002, 129, 53.

39. Veltmaat, J.M.; Relaix, F.; Le, L.T.; Kratochwil, K.; Sala, F.G.; van Veelen, W.; Rice, R.; Spencer-Dene, B.; Mailleux, A.A.; Rice, D.P.; et al. Gli3-mediated somitic Fgf10 expression gradients are required for the induction and patterning of mammary epithelium along the embryonic axes. Development 2006, 133, 2325.
40. Walterhouse, D.O.; Lamm, M.L.; Villavicencio, E.; Iannaccone, P.M. Emerging roles for hedgehog-patched-Gli signal transduction in reproduction. Biol. Reprod. 2003, 69, 8.
41. Dai, P.; Akimaru, H.; Tanaka, Y.; Maekawa, T.; Nakafuku, M.; Ishii, S. Sonic Hedgehog-induced activation of the Gli1 promoter is mediated by GLI3. J. Biol. Chem. 1999, 274, 8143.
42. Ikram, M.S.; Neill, G.W.; Regl, G.; Eichberger, T.; Frischauf, A.M.; Aberger, F.; Quinn, A.; Philpott, M. GLI2 is expressed in normal human epidermis and BCC and induces GLI1 expression by binding to its promoter. J. Investig. Dermatol. 2004, 122, 1503.
43. Hatsell, S.J.; Cowin, P. Gli3-mediated repression of Hedgehog targets is required for normal mammary development. Development 2006, 133, 3661.
44. Kleinberg, D.L.; Wood, T.L.; Furth, P.A.; Lee, A.V. Growth hormone and insulin-like growth factor-I in the transition from normal mammary development to preneoplastic mammary lesions. Endocr. Rev. 2009, 30, 51.
45. Heckman, B.M.; Chakravarty, G.; Vargo-Gogola, T.; Gonzales-Rimbau, M.; Hadsell, D.L.; Lee, A.V.; Settleman, J.; Rosen, J.M. Crosstalk between the p190-B RhoGAP and IGF signaling pathways is required for embryonic mammary bud development. Dev. Biol. 2007, 309, 137.
46. Hens, J.R.; Dann, P.; Zhang, J.P.; Harris, S.; Robinson, G.W.; Wysolmerski, J. BMP4 and PTHrP interact to stimulate ductal outgrowth during embryonic mammary development and to inhibit hair follicle induction. Development 2007, 134, 1221.
47. Wysolmerski, J.J.; Philbrick, W.M.; Dunbar, M.E.; Lanske, B.; Kronenberg, H.; Broadus, A.E. Rescue of the parathyroid hormone-related protein knockout mouse demonstrates that parathyroid hormone-related protein is essential for mammary gland development. Development 1998, 125, 1285.
48. Gritli-Linde, A.; Hallberg, K.; Harfe, B.D.; Reyahi, A.; Kannius-Janson, M.; Nilsson, J.; Cobourne, M.T.; Sharpe, P.T.; McMahon, A.P.; Linde, A. Abnormal hair development and apparent follicular transformation to mammary gland in the absence of hedgehog signaling. Dev. Cell 2007, 12, 99.
49. Howard, B.; Panchal, H.; McCarthy, A.; Ashworth, A. Identification of the scaramanga gene implicates Neuregulin3 in mammary gland specification. Genes Dev. 2005, 19, 2078.
50. Hardy, K.M.; Booth, B.W.; Hendrix, M.J.; Salomon, D.S.; Strizzi, L. ErbB/EGF signaling and EMT in mammary development and breast cancer. J. Mammary Gland Biol. Neoplasia 2010, 15, 191.

51. Wansbury, O.; Panchal, H.; James, M.; Parry, S.; Ashworth, A.; Howard, B. Dynamic expression of Erbb pathway members during early mammary gland morphogenesis. J. Investig. Dermatol. 2008, 128, 1009.
52. Tidcombe, H.; Jackson-Fisher, A.; Mathers, K.; Stern, D.F.; Gassmann, M.; Golding, J.P. Neural and mammary gland defects in ErbB4 knockout mice genetically rescued from embryonic lethality. Proc. Natl. Acad. Sci. USA 2003, 100, 8281.
53. Harris, H.A.; Albert, L.M.; Leathurby, Y.; Malamas, M.S.; Mewshaw, R.E.; Miller, C.P.; Kharode, Y.P.; Marzolf, J.; Komm, B.S.; Winneker, R.C.; et al. Evaluation of an estrogen receptor-β agonist in animal models of human disease. Endocrinology 2003, 144, 4241.
54. Davenport, T.G.; Jerome-Majewska, L.A.; Papaioannou, V.E. Mammary gland, limb and yolk sac defects in mice lacking Tbx3, the gene mutated in human ulnar mammary syndrome. Development 2003, 130, 2263.
55. Bamshad, M.; Lin, R.C.; Law, D.J.; Watkins, W.C.; Krakowiak, P.A.; Moore, M.E.; Franceschini, P.; Lala, R.; Holmes, L.B.; Gebuhr, T.C.; et al. Mutations in human TBX3 alter limb, apocrine and genital development in ulnar-mammary syndrome. Nat. Genet. 1997, 16, 311.
56. Phippard, D.J.; Weber-Hall, S.J.; Sharpe, P.T.; Naylor, M.S.; Jayatalake, H.; Maas, R.; Woo, I.; Roberts-Clark, D.; Francis-West, P.H.; Liu, Y.H.; et al. Regulation of Msx-1, Msx-2, Bmp-2 and Bmp-4 during foetal and postnatal mammary gland development. Development 1996, 122, 2729.
57. Satokata, I.; Ma, L.; Ohshima, H.; Bei, M.; Woo, I.; Nishizawa, K.; Maeda, T.; Takano, Y.; Uchiyama, M.; Heaney, S.; et al. Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. Nat. Genet. 2000, 24, 391.