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Hedgehog Signaling in the Normal and Neoplastic Mammary Gland

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Abstract

The hedgehog signal transduction network is a critical regulator of metazoan development. Inappropriate activation of this network is implicated in several different cancers, including breast. Genetic evidence in mice as well as molecular biological studies in human cells clearly indicate that activated signaling can lead to mammary hyperplasia and, in some cases, tumor formation. However, the exact role(s) activated hedgehog signaling plays in the development or progression of breast cancer also remain unclear. In this chapter, we review recent data regarding the mechanism(s) by which the hedgehog network may signal in the mammary gland, as well as the data implicating activated signaling as a contributing factor to breast cancer development. Finally, we provide a brief update on the available hedgehog signaling inhibitors with respect to ongoing clinical trials, some of which will include locally advanced or metastatic breast cancers. Given the growing intensity with which the hedgehog signaling network is being studied in the normal and neoplastic mammary gland, a more complete understanding of this network should allow more effective targeting of its activities in breast cancer treatment or prevention.

Introduction

The *Hedgehog (Hh)* gene was originally discovered in a screen for genes affecting segment polarity in *Drosophila* larvae (1). Since its discovery, and the discovery of its mammalian orthologs, the hedgehog signaling network has been shown to regulate many of the major developmental processes in both invertebrate and vertebrate species. Recent analysis of two of its main constituent genes suggests that the network might have evolved from existing lipid homeostasis pathways (2). The Hh network plays important roles in the growth, patterning and morphogenesis of a variety of tissues in several different organisms (3). In mammals, this network is known to pattern the limb and the dorso-ventral axis of the neural tube, to stimulate proliferation of neural precursor cells, and to regulate the hair follicle cycle, among others (4). Hh signaling has also been implicated, in conjunction with other major developmental networks like those of Wnt and Notch, in the maintenance and self-renewal of neural, hematopoetic, gut, skin, and mammary gland adult stem cells (5–7). The

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goal of this review is to discuss the role of the Hh network in mammary gland development and breast cancer and to provide a summary of efforts to target hedgehog signaling as a potential therapeutic approach for treatment or prevention of breast cancer.

Mammalian Hh Signaling

Mammalian Hh signaling generally occurs between a signaling cell and a receiving cell, although there are reports of autocrine signaling as well (e.g. (8–10)). Mammals have three ligands: Sonic hedgehog (Shh), Desert hedgehog (Dhh), and Indian hedgehog (Ihh). The Hh proteins are produced and autocatalytically processed in the signaling cell, and released as a palmitoylated and cholesterol modified Hh ligand with the help of the Dispatched (Disp) protein. These two lipid modifications are thought to be required for proper diffusion of the ligand to neighboring cells. Once the ligand is secreted by the signaling cell, it binds the Patched (Ptch) family of twelve-pass transmembrane receptors (Ptch1 or Ptch2) on the receiving cell. When no ligand is bound, the Ptch receptor is thought to inhibit Smoothened (Smo), the network's main signal transducer, catalytically. Smo inhibition allows the phosphorylation and cleavage of two members of the Gli-family of transcription factors (Gli2 and Gli3). This cleavage is mediated by a complex of kinases, and the scaffold protein Suppressor of Fused (SuFu), which phosphorylate the Gli proteins and target them for processing. The cleaved or repressor forms of Gli can then translocate into the nucleus and block transcription of target genes.

Upon ligand binding, the inhibition of Smo by Ptch proteins is relieved, and the signal is transmitted via Smo to inhibit the phosphorylation and cleavage of Gli family transcription factors. The full-length Gli proteins can then translocate into the nucleus and activate transcription of target genes. Of the three Gli family proteins (Gli1, Gli2, Gli3), Gli1 acts exclusively as a transcriptional activator since it lacks the proteolytic cleavage site present in Gli2 and Gli3, whereas Gli2 and Gli3 can act as activators or repressors depending on whether they are cleaved or remain full length. In-vivo data suggests that Gli3 is found primarily in its repressor form (11–13).

Recently several other proteins have been implicated in mammalian hedgehog signaling, including Commodo (Cdo), Brother of Commodo (Boc), Growth Arrest Specicfic-1 (Gas1), and Hedgehog Interacting Protein (Hip) all bind Hh ligands. These proteins can modulate Hh signaling either by sequestering the ligand, in the case of Hip, or by facilitating the binding of the ligand to the Ptch family of receptors as is the case of Cdo, Boc, and Gas1 (14–17).

Recently, the ability to activate hedgehog signaling in mammalian cells has been linked to the presence of a primary cilium on the receiving cell. As such, proteins involved in cilium formation and intraflagellar transport (IFT) have recently been shown to play vital roles in Hh signaling. Recently, mouse IFT proteins have been shown to be required for Gli activator and Gli repressor functions, and mice lacking these proteins exhibit Hh loss-of-function phenotypes (18, 19). *Drosophila* has homologs to IFT components as well, yet they do not seem to be required for Hh signaling since flies lacking these components do not exhibit Hh loss-of-function phenotypes (20–22). This evidence coupled to the recent localization of

mammalian Hh network members to cilia (18, 23), gives strong support to the theory that cilia are important in promoting vertebrate Hh signaling. Recent work aimed at deciphering the mechanism of cilia involvement has identified that the localization of Ptch1 and Smo to cilia is mutually exclusive. Ligand binding results in the internalization of ligand bound Ptch1 and the subsequent translocation of Smo to the primary cilia where activation of Gli proteins can then take place (18, 24, 25). The role of primary cilia in hedgehog signaling is reviewed in greater detail in Wong et al. 2008 (26).

Non-Canonical Hh Signaling

In addition to the canonical mechanism of signal transduction in which ligand binding activates a downstream transcriptional response, several types of non-canonical hedgehog signaling have been identified. These include different roles for Hh network members, signaling that does not lead to activation of transcription, and ligand-independent effects of the downstream transcriptional activators.

In addition to transducing the hedgehog signal, the Ptch receptor can also function to sequester the hedgehog ligand and restrict its free range of diffusion (27, 28). Additionally, the Ptch receptor has been found to act as a “dependence receptor” in cell types that require the presence of ligand-bound receptor in order to survive. In these cells, unbound Ptch receptor leads to the initiation of the apoptotic cell death pathway (29–31). Ptch1 was also found to regulate the cell cycle by controlling the transition from G2 to M phase. Ptch1 was found to interact specifically with constitutively phosphorylated cyclin B1 and to affect its sub-cellular localization by sequestering it in the cytoplasm. Upon Shh ligand addition, this interaction was disrupted allowing cyclin B1 to localize to the nucleus (32). More recently, a group identified Ptch1 as a tumor suppressor and “gatekeeper” of cell cycle progression. In a skin-specific Ptch1 loss-of-function model that led to basal cell carcinoma type lesions, nuclear accumulation of cyclin D1 and cyclin B1 were observed (33). These data suggest that ligand-dependent Hh signaling may elicit effects on a particular cell type or tissue without necessarily requiring a Gli-mediated transcriptional response.

Smoothened is a seven-pass trans-membrane protein that acts as the Hh network’s main signal transducer. It is a non-redundant member of the mammalian signaling network and thus serves as a rate-limiting component. Smo shares structural and evolutionary homology with other G-protein-coupled receptors (GPCRs) such as rhodopsin and β -adrenergic receptor, and is most closely related to the frizzled proteins that mediate Wnt signaling. However, until recently, evidence to support a role for heterotrimeric G-proteins in activated hedgehog signaling has been minimal (34–36).

GPCRs are seven-helix membrane spanning cell-surface receptors that signal through GTP binding and hydrolysis by heterotrimeric G proteins to stimulate or inhibit the activity of a set of downstream enzymes. GPCRs are involved in developmental processes including cardiogenesis, development of epithelial derived structures, and neuronal development among others. The Wnt-Frizzled signaling pathway has been shown to transduce signal via heterotrimeric G proteins in organisms ranging from *Drosophila* to vertebrates. (37)

Evidence for non-canonical hedgehog signaling via Smo-coupled heterotrimeric G protein activation is beginning to come to light. A study by Riobo's group first demonstrated functional interactions between mammalian Smo and selected G_α subunits primarily of the $G_{\alpha i}$ family (38). This study indicated that Smo can couple with G_{i1}, G_{i2}, G_{i3}, G_0, G_z, and G_{15} in Sf9 cells. Four of these G_α subunits (G_{i1}, G_{i2}, G_{i3}, G_0) are irreversibly inhibited by pertussis toxin (PTX)-mediated ADP-ribosylation. In contrast, Smo could not couple with G_s, G_q, G_{qzIc}, G_{qG66D}, $G_{qG66Dx5}$, $G_{12,}$ G_{13}, or G_{16}, although a different study found that $G_{12,}$ and G_{13} were involved in mediating the Shh-Smo response in neuroblastoma cells (39).

These studies are complemented by other work showing that signaling via Smo is enhanced by catalytically active G protein-coupled receptor kinase 2 (GRK2), but not catalytically inactive GRK2. GRK2 activity promoted association of Smo with β-arrestin-2 (40, 41). These activities of β -arrestin-2 and GRK2 contrast in their interactions with most, but not all, other G protein-coupled receptors where they typically function to desensitize cells to ligand stimulation (42). G protein coupling by Smo could be genetically separated from Gli activation using a truncated Smo protein (43), offering the possibility that these two functions might be uncoupled *in vivo* under certain conditions.

Additionally, G protein-coupled receptor kinase2 (GPRK2) was found to participate in hedgehog signaling in *Drosophila*, indicating the possibility that Smo function as a GPCR might be evolutionarily conserved (44). *Drosophila* Smo was found to interact with $G\alpha_i$, and this interaction proved essential for Hh signal transduction (45).

Recently, Shh was found to mediate axon guidance in a Smo-dependent and Gli independent manner via activation of Src family kinases (46). Additionally, Hh ligands were found to activate a pro-angiogenic response in endothelial cells in a Gli-independent manner. Interestingly, these effects could be suppressed by PTX treatment suggesting that a GPCR function for Smo is essential for signaling (47).

Non-canonical Smo-independent signaling has also been suggested for the Gli family of transcription factors. Although previously believed to act solely as the transcriptional activators of the Hh network, emerging evidence suggests that these transcription factors are regulated by other signaling networks including the TGF-β network (48–51).

Targets of Hh Signaling

The Hh signaling network has been known to influence proliferation, growth, differentiation, and patterning of different tissues in a variety of organisms. Several genes are considered targets of Hh signaling including the Gli1 transcription factor, the Ptch receptor and the Hedgehog interacting protein (Hip). The latter two are thought to be part of a negative feedback loop. Most of the targets of this signaling network have been identified from studies in *Drosophila* (4). Recent publications provide increasing evidence of the divergence between vertebrate and invertebrate Hh signaling mechanisms (52, 53). Furthermore, gene profiling studies of the Hh signaling response in mammalian systems have demonstrated that target genes vary dramatically depending on the tissue, ligand used to activate the network,

and the developmental stage of the tissue assayed (54–56). This lack of a unified set of mammalian targets of Hh signaling remains a significant stumbling block in the field.

Hh Signaling in Cancer

The first evidence for a role of the Hedgehog network in cancer came from study of patients with Gorlin's syndrome. Individuals with this disease carry inherited loss-of-function mutations in the Ptch1 gene, and are strongly predisposed to basal cell carcinoma (BCC) (57, 58). Since then, activated Hedgehog signaling has been implicated in medulloblastoma, glioblastoma, rhamdomyosarcoma, and melanoma as well as in breast, pancreatic, lung, prostate, gastrointestinal, and hematological cancers, among others. The role of the hedgehog network in these malignancies is well reviewed in (59).

Initially, most aberrant Hedgehog activation was thought to occur either in a ligand-independent manner, as in the case of BCC, or in a ligand-dependent autocrine signaling loop intrinsic to the tumor. Recently, new evidence has suggested that in several tumor types, including pancreatic and gastrointestinal malignancies, activated Hedgehog signaling is observed in a paracrine manner as a function of tumor-derived ligand production in the adjacent stroma and not the tumor itself (60–62).

A requirement for primary cilia during tumor development is not clear. Since cilia appear to be required for the processing of both the activator and repressor forms of Gli, the role of primary cilia in tumorigenesis may be context dependent. There is evidence indicating that cilia can both mediate and suppress Hh network dependent tumorigenesis (63).

The Role of the Hedgehog Network in Breast Development and Cancer

Mammary Gland Development

Development of the mouse mammary gland and the human breast is remarkably similar. In both species, gland development is a progressive process that begins during embryonic life with the formation of a rudimentary ductal tree, which after this initial growth remains fairly quiescent from birth until puberty. During puberty the rudimentary ducts present at birth begin to grow and elongate as secondary and tertiary ducts spearheaded by rapid and invasive growth of the terminal end bud (TEB). The TEB is a bulb-shaped structure consisting of multiple layers of rapidly dividing, immature epithelial cells. Upon reaching the edges of the available mammary fat pad, TEB structures regress leaving only differentiated ducts. In human, a certain level of alveolar development also occurs due to the presence of a luteal phase of the menstrual cycle. In contrast, most commonly used mouse strains lack a luteal phase to their estrus cycles and thus lack appreciable alveolar development in virgin animals. With pregnancy alveolar development reaches its full potential, followed by the production and secretion of milk during lactation (64). Upon weaning and in response to the halt of the suckling stimulus, the mammary gland begins the involution process characterized by extensive apoptosis and remodeling that culminates in a mammary ductal tree that morphologically resembles the adult virgin (65).

A majority of what we know about human breast development is limited to morphological studies. The major insights into what genes and signaling networks are involved in breast development come from the study of model organisms, primarily mice (Table 1).

Hh Network Expression and Function in Mammary Gland Development

The hedgehog ligands are expressed in mouse mammary epithelium at several stages of development. Ihh and Dhh are detectable in the pubertal gland by in-situ hybridization and Ihh levels are upregulated during pregnancy and lactation (66). Embryonic tissue transplantation of individual knockouts of the Ihh and Shh ligands showed no overt developmental phenotype. The Dhh knockout has not been carefully analyzed for a mammary phenotype, but homozygous females are able to feed their pups successfully suggesting that no major impairments in mammary gland function exist in these knockout mice (67). It is important to recognize that hedgehog ligands can compensate for one another functionally, thus, the lack of phenotype in single gene knockouts might be explained by functional redundancy.

Ptch1 is expressed in both epithelial and stromal compartments of the mouse mammary gland. Loss-of-function studies using several different Ptch1 mutants have demonstrated a role for Ptch1 for normal patterning and elongation of the mammary ductal tree (66, 68, 69). Loss of Ptch1 function has been associated with forfeiture of quiescence and expansion of a progenitor cell pool via differential regulation of the TP63 promoter (68). Transplantation studies revealed that Ptch1 was required in both mammary epithelium and stroma for appropriate mammary gland development (66, 69). Additionally, a role for Ptch1 in pituitary function that influenced ductal elongation was also found (69). Ptch2, although detectable by gene expression analysis in mature virgin mice, has not been studied in the context of mammary gland development. Ptch2 knockout mice are viable, and no defects in lactation were reported in this model, possibly due to compensation by Ptch1 (70).

Expression and function analyses of the Gli family of transcription factors in mammary epithelium have yielded somewhat different results. Using a Gli1-lacZ knock-in reporter, Gli-1 was only detected in lymphatic vessels in both the embryonic and adult mammary gland (71). Consistent with lack of expression in mammary epithelium, Gli1 loss-of-function had no phenotype as a single gene mutation. In contrast, targeted overexpression of Gli1 in mammary epithelium led to impaired lobuloalveolar development and lactation defects in transgenic female mice (72).

With respect to Gli2 expression, Gli2 was detected by in situ hybridization and localized exclusively to the periductal stroma during virgin development. However, expression became both epithelial and stromal during pregnancy and lactation (73). Transplantation analysis of embryonic mammary glands derived from a targeted disruption mutant for Gli2 revealed that Gli2 function is required for normal mammary gland development. However, transplantation of epithelial fragments from homozygous mutant animals into cleared fat pads of immune compromised mice failed to recapitulate the dysplastic ductal phenotypes observed in whole gland transplantation, indicating that Gli2 functions primarily in the mammary stroma to affect mammary epithelial cell behavior (73). In a different study (71), Gli2 and Gli3 were found to be expressed in stromal cells and in myoepithelial cells after

pregnancy. Gli3 was additionally found in luminal epithelial cells, and was shown to be essential for proper formation of the embryonic mammary buds. This study found no requirement for either Gli1 or Gli2 in embryonic mammary gland development, and proposed that the Hedgehog network must remain inactive for appropriate embryonic and pubertal development of the mammary gland (71).

The hypothesis that the hedgehog network must remain inactive throughout mammary gland development has not yet been tested formally by disruption of *Smo*. These studies are ongoing in our laboratory using conditional Cre-recombinase-mediated *Smo* disruption. However, overexpression of a constitutively active form of Smoothened (SmoM2) under the control of the mouse mammary tumor virus (MMTV) promoter has been performed and led to increased proliferation and hyperplasia of the mammary ductal tree (74). These phenotypes were accompanied by depletion of the regenerative stem cell pool, and expansion of a population of cells capable of anchorage-independent growth as mammospheres in non-adherent culture conditions. Together with the observation that Ptch1 disruption increased the progenitor pool, these data have suggested the hypothesis that activated hedgehog signaling promotes exit from the stem cell pool, but persistence of a relatively undifferentiated progenitor cell pool.

Role of primary cilia in mammary gland hedgehog signaling

Until recently, the existence of cilia in mammary epithelium had not been carefully examined in mouse or human tissue. McDermott et al. examined the distribution of cilia in the mouse mammary gland and found that epithelial, myoepithelial, and stromal cells all contained cilia. Interestingly, cilia distribution in epithelial cells was highest during pubertal development and less abundant in mature adult tissues. Using a mouse model with a ciliary defect they also noticed a reduction in branching morphogenesis in the mammary ductal tree in mice in which cilia were absent. This was accompanied by an increase in Wnt signaling and a decrease in canonical Hh signaling, at least as defined by reduced *Gli1* mRNA expression (75).

The Hh Network in Breast Cancer

A potential role for activated hedgehog signaling in breast cancer was postulated almost immediately upon identification of mutations in Ptch1 associated with Gorlin's syndrome and BCC. However, Gorlin's syndrome patients do not show increased risk of breast cancer. Thus, significant evidence supporting such a role was not forthcoming until recently (reviewed in (76, 77)).

With respect to genetic alterations in hedgehog network genes, analysis of mutations in breast cancers has thus far shown little evidence that mutation of hedgehog signaling genes are common. For example, while a small early study identified *Ptch1* mutations in 2 of 7 human breast cancers (78), similar analyses in larger cohorts failed to identify these mutations (79, 80). More recent genomic sequencing efforts identified 3 missense mutations in the *Gli1* gene in 11 breast cancer samples examined, but the functional significance of these mutations remains untested (81). Provocatively, a *Ptch1* polymorphism was linked to increased breast cancer risk associated with oral contraceptive use (82).

With respect to larger genetic changes, array comparative genomic hybridization (CGH) analyses indicate that genomic loss at the *Ptch1* locus was the fourth most commonly detected change among the tumor suppressor genes identified in the study, occurring in 19% of human breast cancers, and 33% of breast cancer cell lines (83). Amplification of the *Gli1* gene has also been demonstrated (84).

Data related to expression of hedgehog network genes in human breast cancer are currently ambiguous. An early immunohistochemical staining study suggested that hedgehog signaling is activated in a majority of human invasive breast cancers based on ectopic expression of Ptch1 and Gli1 (85), which were not detected in normal tissue. A second study in at least 8 patient matched samples showed Shh, Ptch1 and Gli1 expression (86) in both normal and cancer tissue. In this study, expression of Shh was increased in the epithelium of 5 of 8 samples, with an increase in Gli1 expression in cancerous epithelium in 4 of 8 samples examined. There was also an increase in Ptch1 expression in 3 of 9 samples examined. Gli protein and mRNA expression were positively correlated with one another. However, Ptch1 protein and mRNA expression were not. *Smo* mRNA levels were significantly higher than normal in 4 of 10 samples examined. These two studies are in general agreement with a recent publication (87), which analyzed 21 normal breast samples and 121 invasive ductal carcinomas by immunohistochemistry for expression of Ihh, Ptch1, Smo, Gli1, Gli2, and Gli3. For all six genes, expression was higher in invasive ductal carcinoma relative to normal breast epithelia with several correlations between expression of individual genes with clinical biomarkers and behaviors.

These studies conflict in some specifics with other published analyses. For example, using a panel of normal, ductal carcinomas in situ (DCIS), and invasive breast cancer (IBC) samples (74), Ptch1 was detectable at moderate levels throughout the epithelium, and in isolated stromal cells of the normal breast. These patterns were consistent with expression patterns in the mouse mammary gland by both immunolocalization and in situ hybridization (66, 74). However, Ptch1 expression was decreased or absent in ~50% of ductal carcinoma in situ (DCIS) and invasive breast cancers (IBC). With respect to Smo, expression was undetectable in normal breast, but Smo was ectopically expressed in ~70% of DCIS and ~30% of IBC (74). Expression of Ptch1 and Smo did not correlate with histological grade (DCIS only) or with expression of any clinically relevant marker tested (estrogen receptor, ErbB2, p53). Data related to Ptch1 in this study were in complete agreement with an independent study by Wolf et al (88) in which they demonstrated epigenetic silencing of the *Ptch1* gene in MCF7 cells, as well as reduced protein expression in clinical specimens. Increased Smo protein expression in human breast cancer was generally consistent with the Q-PCR results of Mukherjee et al. (86) which showed increased mRNA expression in ~40% of their samples.

Functional analyses of hedgehog network genes offer hints as to how changes in hedgehog signaling may contribute to breast cancer development or progression. In human cells, activated hedgehog signaling has been associated with regulation of CD44+;CD24- breast cancer stem cells (89, 90). Genetic knockdown of *Gli1* and *Gli2* resulted in reduced mammosphere formation, while overexpression of *Gli2* in human cells led to hyperplasia. Indeed, in MDA-MB-231 cells, *Gli2* was shown to be required for osteolytic behavior in a metastasis model (91).

Despite the development of hyperplasia and dysplasia in several genetically engineered mouse models (66, 69, 73, 74), long-term tumor formation studies in *Ptch1* heterozygotes did not show increased frequency of mammary tumors (92). Similarly, using transgenic mice expressing *SmoM2* under the control of the MMTV promoter, long-term studies ongoing in our laboratory have thus far failed to show an increase in tumor formation in this model. In contrast with these data, overexpression of *Gli1* did result in tumor development (93) with multiple histopathologies and expression of basal cell type markers. One potential explanation for the difference in tumor formation potential of *Gli1* overexpression versus either *Ptch1* loss or *Smo* overexpression (both predicted to activate signaling) is the observation that neither *Ptch1* loss nor *Smo* overexpression led to the anticipated increase in *Gli1/2* expression observed in BCC and other hedgehog related cancers (74).

Clinical implications of activated hedgehog signaling in breast cancer

In recent years, a number of hedgehog signaling antagonists have been identified and characterized (Table 2) (exhaustively reviewed in (94) and references therein). Antagonists include a group of plant-derived steroidal alkaloids (e.g. cyclopamine and jervine) first identified as potent teratogens in sheep, rodents, and other vertebrates (95–97). Such compounds act by direct binding to Smo to inhibit ligand responsiveness and downstream signaling (98–100). In addition to these naturally occurring antagonists, several other hedgehog signaling agonists and antagonists have been identified or synthesized that target either the Shh ligand, Smo, or the downstream Gli transcription factors (Table 2).

In preclinical studies, cyclopamine and CUR0199691 have been used in vitro to treat various breast cancer cell lines (85, 86, 101). Results are generally consistent across studies, with cyclopamine doses of 10uM or higher leading to significant inhibition of cell growth via both reduction of proliferation and induction of apoptosis. However, the specificity of these compounds at the doses required for inhibition remains an open question given that these two compounds showed activity against different sets of cell lines, and activity did not correlate with detectable expression of Smo mRNA, nor did activity correlate with the ability of cell lines to respond to treatment with recombinant Shh ligand (101). Preclinical studies in mice using Smoothened inhibitors have shown promising effects in prevention of metastases from pancreatic cancer (102), as well as inhibition of tumor growth in medulloblastoma (103).

Several of the selected hedgehog signaling modulators listed in Table 2 have entered, or are poised to enter, clinical trials (www.clinicaltrials.gov). The Genentech compound GCD-0449 has completed a phase I clinical trial in patients with locally advanced or metastatic basal cell carcinoma with measurable responses in 29/33 patients (including 2 complete responses) with no dose-limiting toxicities (104, 105). This compound is now in phase II trials in patients with a variety of cancers including advanced or metastatic basal cell carcinoma, pancreatic cancer, gastric cancers, colorectal, and ovarian cancers. In advanced breast cancer, GCD-0449 is being investigated in combination with a gamma secretase inhibitor (RO4929097) to block Notch signaling.

Compounds from other companies are in earlier stages of the approval process. The Pfizer Smo inhibitor PF-04449913 has entered a phase I clinical trial in patients with hematological malignancies including CML for use either alone or in combination with Dasatinib (a c-src inhibitor). The BMS compound BMS-833923 (XL139) has entered a phase I clinical trial for patients with BCC. The Infinity compound IPI-926 has entered A Phase 1 Study in patients with advanced and/or metastatic solid tumor malignancies. Similarly, the Novartis compound LDE225 is also in Phase I trials in patients with advanced solid tumors as well as basal cell carcinoma and medulloblastoma. Given that advanced solid tumors would include breast, results from these phase I and phase II trials should be informative with respect to potential efficacy against advanced breast cancers.

Concluding remarks

Despite the recent upsurge in interest in the hedgehog signaling network in breast cancer, there are still major deficiencies in our understanding of exactly how hedgehog signal transduction occurs in mammary epithelium at the molecular level. There also remain major deficiencies in our knowledge of gene expression patterns and in our understanding of the function of hedgehog network genes in mammary gland development and disease development. Nevertheless, the availability of new small molecule inhibitors, particularly those targeting Smo, offer the possibility that these agents may be useful clinically for the treatment (or prevention) of breast cancer either alone or, more likely, in combination with other systemic therapies. With ongoing clinical trials now including advanced breast cancers, an indication of the clinical usefulness of hedgehog inhibitors against breast cancer should not be far off.

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Table 1

Analyses of Mammary Gland Phenotypes in Hedgehog Pathway Mouse Models

	Mouse model	Effect on Pathway	Mammary Gland Phenotype	Epithelial/Stromal Role	Reference
Ligands	*Shh*$^{-/-(Rescue\ Transplants)}$	decreased activity	No overt phenotype	–	{66}
	Dhh$^{-/-(Rescue\ Transplants)}$	decreased activity	No overt phenotype	–	{66}
Receptors	*Ptch1*$^{tm1Mps/+}$	increased activity	aberrant TEBs, ductal dysplasia	both	{66}
	Ptch1^{Mes}	?	ductal dysplasia	both	{68}
	Ptch1$^{lzk1/+}$	increased activity	no embryonic MG phenotype	–	{70}
	MMTV-Cre; Ptch1$^{fl/fl}$	increased activity	dilated ducts	epithelial	{68}
Transducers	*MMTV-SmoM2*	increased activity	increased branching and budding	epithelial	{73}
Effectors	*MMTV-Gli1*	increased activity	impaired lobuloalveolar development	epithelial	{71}
	Gli1$^{lzk1/lzk1}$	decreased activity	no overt phenotype	–	{70}
	Gli3$^{ext/+}$	increased activity	aberrant mammary placode formation	both	{70}
	Gli2$^{lzki/lzki}$	decreased activity	no overt phenotype	–	{70}
	Gli2$^{-/-(Rescue\ Transplants)}$	decreased activity	aberrant ductal morphogenesis	stromal	{72}

Table 2

Selected Small Molecule Modulators of Hedgehog Signaling

Activity	Name	Target	Company	Usage	A.K.A
Agonists	*Purmorphamine*	SMO		Basic/Preclinical	
	SAG	SMO		Basic/Preclinical	
Antagonists	*Cyclopamine*	SMO	Natural product	Basic/Preclinical	
	Jervine	SMO	Natural product	Basic/Preclinical	
	SANT19	SMO		Basic/Preclinical	
	SANT74	SMO		Basic/Preclinical	
	SANT75	SMO		Basic/Preclinical	
	Cur61414	SMO	Curis	Basic/Preclinical	
	Cur0199691	SMO	Curis	Basic/Preclinical	HhAntag691
	GDC-0449	SMO	Curis/Genentech	Clinical	RG3616
	IPI926	SMO	Infinity Pharmaceuticals	Clinical	
	LDE225	SMO	Novartis	Clinical (planned)	
	XL-139	SMO	Bristol-Meyers Squibb	Clinical	BMS-833923
	PF-04449913	SMO	Pfizer	Clinical (planned)	
	unknown	SMO	Amgen		
	unknown	SMO	Eli Lilly		
	unknown	SMO	Takeda		
	GANT58	GLI		Basic/Preclinical	
	GANT61	GLI		Basic/Preclinical	
	Physalin F	GLI		Basic/Preclinical	
	Physalin B	GLI		Basic/Preclinical	
	NMDA298-1	GLI		Basic/Preclinical	
	JK-184	GLI		Basic/Preclinical	
	Robotnikinin	SHH		Basic/Preclinical	
	HPI-1	Unknown			
	HPI-2	Unknown			
	HPI-3	Unknown			
	HPI-4	Unknown			