Breast Cancer RESEARCH

REVIEW

Key signaling nodes in mammary gland development and cancer: β-catenin

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Abstract

β-Catenin plays important roles in mammary development and tumorigenesis through its functions in cell adhesion, signal transduction and regulation of cell-context-specific gene expression. Studies in mice have highlighted the critical role of β-catenin signaling for stem cell biology at multiple stages of mammary development. Deregulated β-catenin signaling disturbs stem and progenitor cell dynamics and induces mammary tumors in mice. Recent data showing deregulated β-catenin signaling in metaplastic and basal-type tumors suggest a similar link to reactivated developmental pathways and human breast cancer. The present review will discuss β-catenin as a central transducer of numerous signaling pathways and its role in mammary development and breast cancer.

Introduction

β-Catenin plays a significant role in the regulation of mammary development and tumorigenesis. At the plasma membrane, β-catenin aids cadherins in maintaining mammary epithelial integrity. In the nucleus, β-catenin regulates gene expression programs that are essential for mammary stem cell biology during mammary development. Loss of β-catenin from cell–cell adhesive junctions or elevation in the cytosol and nucleus can occur independently by numerous routes. Importantly, both events predispose the breast to cancer. In the present article we review pathways relevant to breast that feed into the β-catenin signaling node, the role of β-catenin signaling in normal mammary development, and the effects of deregulating β-catenin on breast cancer.

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Upstream regulators

Cadherin association

Breast epithelium is bilayered, comprising an inner luminal layer and a surrounding basal layer. In normal breast, the majority of β-catenin localizes to cell–cell adherens junctions through association with cadherins and only a minor portion is involved in signaling.

The importance of the cadherin/catenin complex for epithelial integrity and survival has been demonstrated by conditional deletion of E-cadherin. E-cadherin null cells undergo apoptosis but in the absence of p53 these cells survive and recapitulate many features of lobular breast cancer [1-3]. In humans, lobular breast cancer is associated with irreversible E-cadherin loss and transient changes in cadherin expression facilitate metastasis in many ductal breast cancers [4].

Although the consequences of β-catenin deletion have not been experimentally investigated, loss of cadherin-bound β-catenin correlates significantly with poor outcome in breast cancer [5]. This loss can occur in three ways. Factors such as transforming growth factor beta (TGFβ), and some Wnts, reduce catenin localization to adherens junctions by increasing Snail, Slug, Twist, Siah interacting protein (SIP) and WISP3 expression (for a review see [6]), which suppress E-cadherin transcription. Similarly, loss of $p120^{ctn}$ – which prevents cadherin internalization – reduces membrane-bound β-catenin by destabilizing E-cadherin [7]. The major mechanism affecting catenin localization to the plasma membrane, however, involves phosphorylation. Many tyrosine kinases, notably epidermal growth factor receptor/ErbB1/Her1, Met, Abl, Src and Fer, directly regulate cadherin/catenin affinity for one another and are strongly implicated in breast cancer [8-14]. For instance, ErbB2/Her2/c-Neu mutation drives 30% of breast cancer, and increased β-catenin-ErbB2/Her2/c-Neu association has been documented in human infiltrating ductal adenocarcinoma and metastases [15,16]. Recent studies have also revealed that serine/threonine phosphorylation of the N-terminal domain of β-catenin by GSK3β and CK1 kinases plays a significant role in compartmentalizing β-catenin [17]. A further mechanism of cadherin/catenin disruption involves the transmembrane mucin, MUC1, which competes with

cadherins for catenin association [18,19]. MUC1 is overexpressed in 90% of breast carcinomas and parous MMTV-Muc1 mice develop mammary tumors [20].

Canonical Wnt signaling

While cadherin/catenin complexes are critical to mammary integrity, cytosolic and nuclear β-catenin serve a distinct role, transducing signals from multiple pathways into cell-context-specific gene expression patterns that are essential for mammary development. This role has been studied most intensively within the context of canonical Wnt signaling.

Wnts are secreted morphogens that provide positional information to neighboring cells through canonical and noncanonical signaling routes (for recent reviews, see [21,22] – primary references and a detailed description of Wnt pathways can be accessed online: http://www.stanford.edu/~rnusse/wntwindow.html). In brief, the canonical Wnt pathway operates by stabilizing cytosolic β-catenin (Figure 1). In the absence of Wnt signals, a multiprotein destruction complex – including the tumor suppressor adenomatous polyposis coli (APC) and Axin – facilitates serine/threonine phosphorylation of the N-terminal domain of β-catenin, which leads to its ubiquitination and proteosomal destruction. Wnt ligands prevent this sequence of events by binding to Frizzled receptors and signaling through the associated low-density lipoprotein-related proteins, LRP5/6. LRP signaling via Disheveled and Axin inhibits the destruction complex and thus induces cytoplasmic accumulation of β-catenin. This accumulation promotes β-catenin translocation to the nucleus, where it associates with T-cell factor/Lef DNA binding proteins, displaces transcriptional repressors, and recruits B-cell lymphoma-9/legless, pygopus homolog 2 (Pygo2) and other factors to induce cell-context-specific gene expression. Canonical Wnt signaling is highly conserved and, importantly, regulates stem cell dynamics in many tissues, including the breast [23].

Other pathways regulating β-catenin signaling

Although canonical Wnt signaling is the most well-known path to β-catenin stabilization, numerous other signaling pathways – many of which are aberrant in breast cancer – also regulate β-catenin stability and transcriptional activity (Figure 2). For example, the prolyl isomerase Pin1 prevents association of β-catenin with APC, providing a major Wnt-independent stabilization mechanism [24]. Akt and ILK also stabilize β-catenin by inhibiting GSK3β activity. Importantly, the tumor suppressor phosphatase and tensin homolog (PTEN) inhibits both pathways [25,26]. Additionally, members of the p53–DNA damage pathway, Siah-1 (RING domain protein; transcriptional target of p53) and Ebi (F box protein) interact with APC to form an alternative destruction complex that recognizes and destroys β-catenin regardless of its phosphorylation status [27-29]. The p53 homolog, ΔNp63, was shown to be a target of p53-dependent destruction. During cancer, loss of p53 permits the aberrant interaction of ΔNp63 with protein phosphatase (PP2A), which inhibits GSK3β leading to β-catenin stabilization [30]. Finally, critical kinases of the NF-κB signaling pathway (IκB kinase (IKK)α and IKKβ) differentially regulate β-catenin transcription as well as activity [31]. IKKα phosphorylation increases stability and transcriptional activity of β-catenin, while phosphorylation by IKKβ decreases both [31]. In certain cell contexts, Wnts stimulate alternative effectors termed noncanonical pathways that can antagonize the β-catenin pathway (Figure 1). A web of regulatory pathways thus converges on cytosolic β-catenin and becomes transduced into a series of thresholds that exquisitely regulate the timely development and cyclical remodeling of the mammary gland. (See Table 1 for a summary of the phenotypes of transgenic and knockout mice used to study Wnt signaling in mammary development and cancer.)

β-catenin signaling and mammary stem cells

In many epithelia, including breast, Wnt pathway activity is critical for stem cell self-renewal and multipotency [23]. β-Catenin signaling exerts powerful effects on mammary stem cells and progenitor cells. In mice, mammary stem cells reside among differentiated myoepithelial cells of the basal layer and alveolar progenitors are scattered among the differentiated cells of the luminal layer [32,33]. Recent studies have shown that Lrp5 is expressed in the basal epithelium and cells with high expression have a 200-fold greater ability to regenerate a mammary tree when transplanted into cleared mammary fatpads. Conversely, $Lrp5^{-/-}$ cells lack this capability and show senescence in culture, demonstrating that canonical Wnt signaling is a critical for normal mammary stem cell maintenance [34]. Supporting this concept, exogenous Wnt 3A protects mammary stem cells from senescence and the loss of multipotency associated with long-term culture. Furthermore, MECS derived from mice where Wnt signaling is mildly derepressed outcompete wild-type MECS in mammary reconstitution assays [23]. The diverse roles of β-catenin signaling during mammary development, which we will now review, may thus be understood in a unified way when viewed through the lens of the highly conserved role of canonical Wnt signaling in regulating stem cell dynamics.

Wnt/β-catenin signaling during embryonic mammary development

Wnt signaling is essential for specification and morphogenesis of the mammary gland [35]. Canonical Wnt signaling reporters define the mammary lines and later

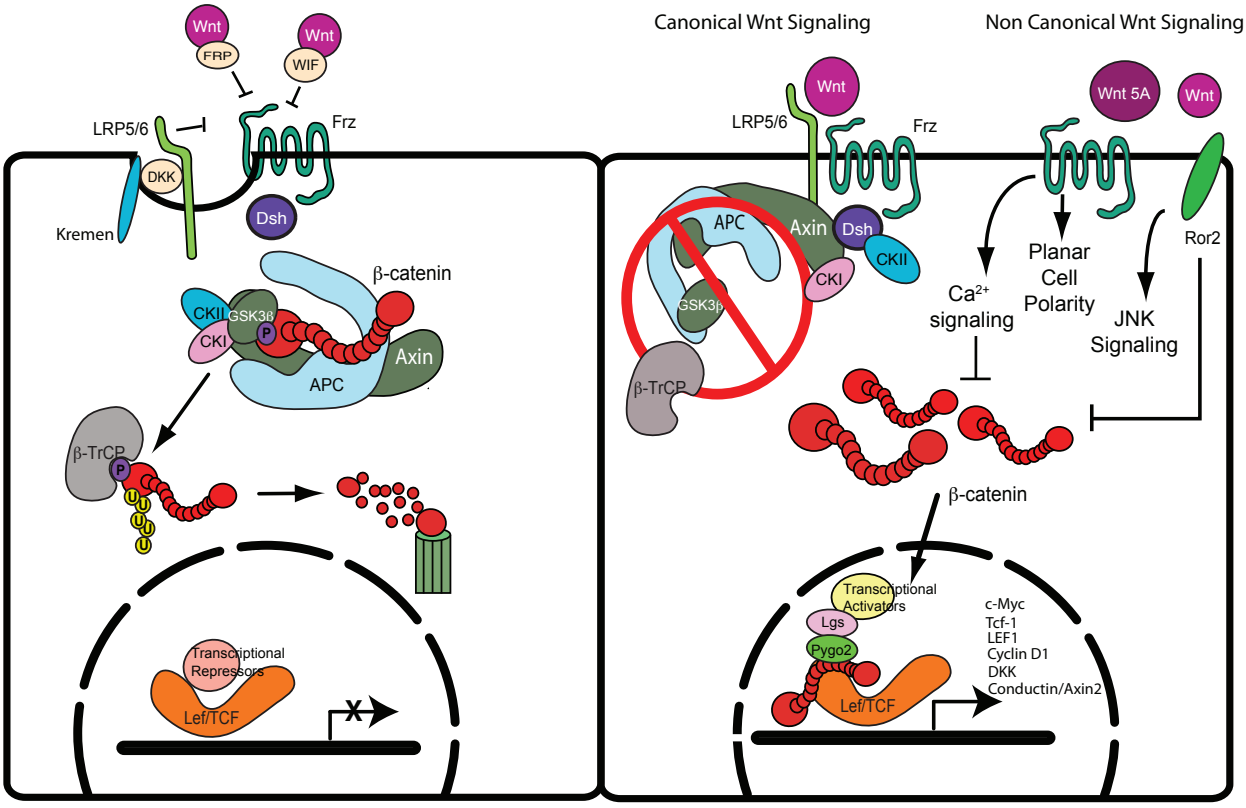

Figure 1. Canonical and noncanonical Wnt signaling pathways. In the absence of Wnt ligand (left panel), β-catenin is bound by a destruction complex within which Axin and adenomatous polyposis coli (APC) serve as scaffolds. Bound β-catenin is phosphorylated by glycogen synthase kinase-3β (GSK3β) and casein kinase 1 (CK1), then ubiquitinated by beta-transducing repeat-containing protein (β-TrCP) and targeted for proteasomal destruction. Binding of Wnt ligand (right panel) inhibits the destruction complex, allowing β-catenin to accumulate in the cytoplasm and translocate to the nucleus, where it binds Lef/T-cell factor (TCF) transcription factors and activates target genes. In addition, Wnt5a can activate noncanonical signaling pathways in cell-context-specific ways. DKK, Dickkopf; FRP, frizzled-related protein; Lgs, legless; LRP, low-density lipoprotein-related protein; Pygo2, pygopus homolog 2; WIF, Wnt inhibitory factor.

become restricted to cells forming placodes [36]. The functional importance of Wnt signaling is demonstrated by experiments showing that Dkk1 suppresses early canonical Wnt signaling, subsequent Wnt10b induction along the mammary line and all placodal development [36]. Targeting other positive acting elements of the Wnt pathway, such as Lrp6, Lrp5, Lef1 and Pygo2, also results in placodal impairments, ranging from loss to reduced size and degeneration, while stimulating β-catenin signaling produces the converse effect – acceleration, expansion and induction of placodes and placodal markers (Wnt10b and T-box transcription factor-3) [37-40]. Candidate activators of early β-catenin signaling include Wnt3, Wnt6 and Wnt10b, which are expressed diffusely throughout the ectoderm as well as a genetic hierarchy of factors, including ventral bone morphogenetic protein-4, dorsal T-box transcription factor-3, neuregulin-3 and somitic Fgf10, which function upstream to define the dorsal–ventral position of Wnt10b and Lef1 induction along the mammary line [35,41-48]. While these upstream factors are body-site specific, Wnt/β-catenin signaling is required universally by all mammary placodes – and indeed by all ectodermal appendages [36]. Downstream targets remain obscure but probably include ectodysplasin-A, which promotes placodal cell fate along the mammary line when overexpressed [49].

Canonical Wnt signaling is also essential for mammary morphogenesis when placodes invaginate to form flask-shaped buds. While recombinant Wnt3a accelerates this process, placodes from Lef1$^{-/-}$ mice remain elevated and degenerate [36,37,50]. Bud invagination is dependent upon mammary mesenchyme specification, which is governed by parathyroid hormone-related peptide (PTHrP). Significantly, mesenchymal expression of canonical Wnt reporters, Lef1 and several target genes is dependent on PTHrP [51-56]. Microarray studies have begun to identify downstream effectors, and β-catenin target genes appear to represent an important module of the PTHrP-induced mammary mesenchyme specification process [57]. Ectodermal β-catenin signaling is also

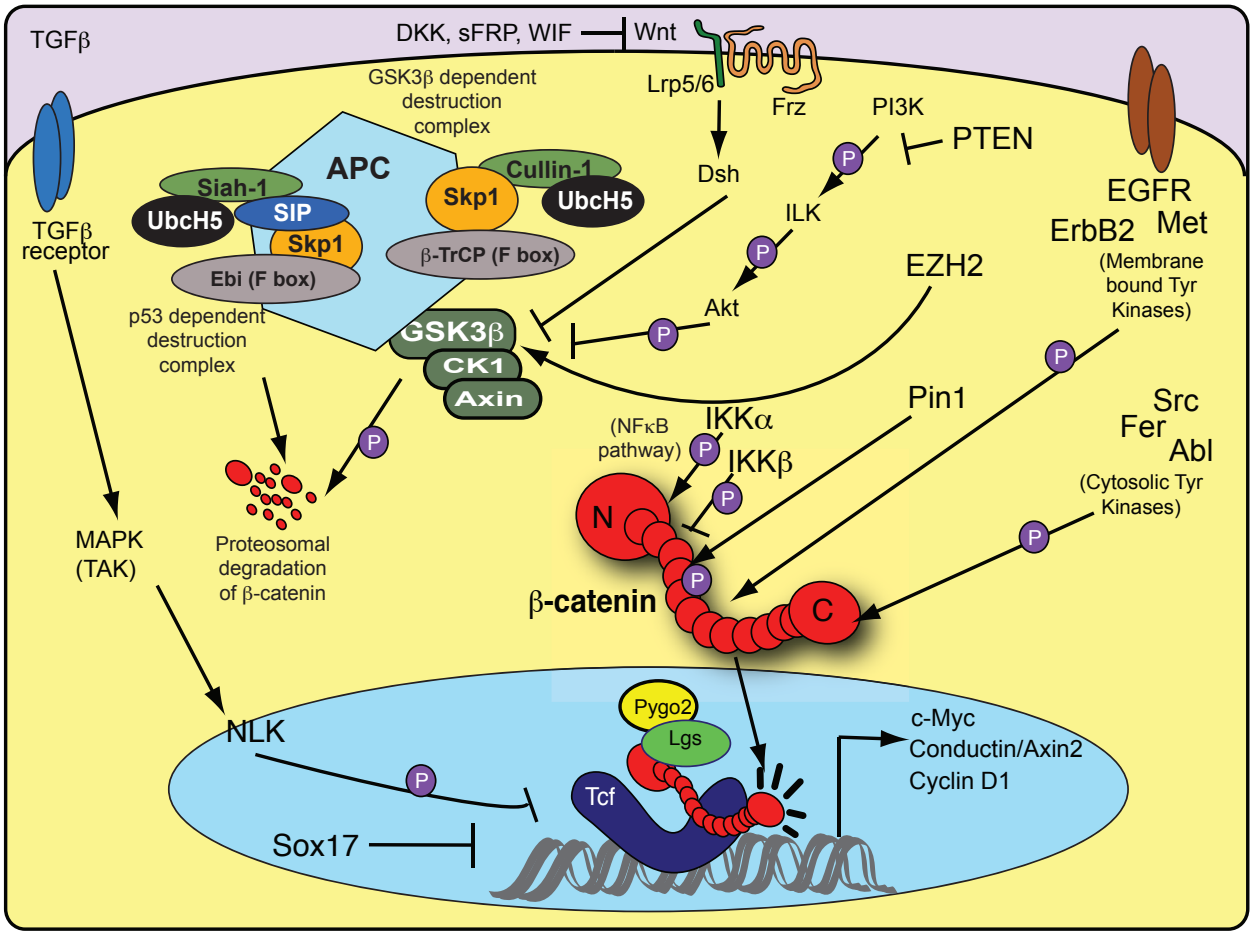

Figure 2. β-Catenin is at the hub of multiple signaling pathways. Many signaling pathways regulate the stability or binding interactions of β-catenin. In the Wnt pathway, glycogen synthase kinase-3β (GSK3β) and casein kinase 1 (CK1) phosphorylate the N-terminal degron sequence of β-catenin to facilitate its destruction. The phosphatidylinositol-3-kinase (PI3K) and phosphatase and tensin homolog (PTEN) pathways also impinge upon β-catenin phosphorylation by regulating GSK3β activity. In addition, p53 induces the degradation of β-catenin through protein interactions involving Seven in absentia homolog 1 (Siah1), Siah interacting protein (SIP) and EBV-induced G-protein coupled receptor (Ebi), resulting in ubiquitination and degradation of β-catenin. Pin1 binds to β-catenin phosphorylated on S246P to prevent its association with adenomatous polyposis coli (APC). In the NF-κB pathway, IκB kinase (IKK)α and IKKβ phosphorylate β-catenin throughout the protein to activate and inhibit transcription, respectively, although the N-terminus is essential for IKKα regulation. Some proteins, such as those within the transforming growth factor beta (TGFβ) pathway and Sox17, regulate β-catenin in the nucleus by modulating its interaction with transcriptional co-activators Tcf/Lef. Other proteins, like enhancer of zeste homolog 2 (EZH2), interact with β-catenin to promote its translocation into the nucleus. A number of tyrosine kinases phosphorylate (both membrane bound and cytosolic) β-catenin to prevent its binding to the cadherin complex at the cell membrane. Src, epidermal growth factor receptor (EGFR), and erythroblastic leukemia viral oncogene-2 (ErbB2) have been shown or implicated to phosphorylate β-catenin on Y654, while Abelson tyrosine kinase (Abl) phosphorylates Y489. β-TrCP, beta-transducing repeat-containing protein; DKK, Dickkopf; Lgs, legless; LRP, low-density lipoprotein-related protein; MAPK, mitogen-activated protein kinase; NLK, Nemo-like kinase; Pygo2, pygopus homolog 2; sFRP, secreted frizzled-related protein; WIF, Wnt inhibitory factor.

important within a population of cells at the sprout tip that express Pygo2, a nuclear partner of β-catenin. Sprout downgrowth and branching are impaired in both Pygo2$^{-/-}$ mice and Lrp6$^{-/-}$ mice [38,39].

β-catenin signaling during pubertal mammary development

Hormones temporally regulate postnatal development of the mammary gland, but their actions are relayed spatially by local paracrine factors. During puberty, estrogen, growth hormone and local IGF stimulate cell proliferation within terminal end buds to produce ductal extension. No direct connection has been established as yet between estrogen and canonical Wnt signaling. Several Wnts peak in expression during puberty, however, and Wnt5a and Wnt7b mRNAs are particularly enriched in terminal end buds and Wnt2 in the surrounding stroma [58-62]. Most reporters of canonical Wnt

Table 1. Mouse models used to study canonical Wnt signaling in mammary development and cancer

Mouse model	Structural modification	Mammary phenotype	References
MMTV-ΔN89-β-catenin	Stabilizing deletion of first 89 amino acids of β-catenin	Expansion $K18^{+}CD29^{hi}$ $49f^{lo}$	[67,75]
	Luminal expression	Precocious alveologenesis	
		Adenocarcinoma	
MMTV-ΔN90-β-catenin	Stabilizing deletion of first 90 amino acids of β-catenin	Hyperplasia	[90]
	Luminal expression	Adenocarcinoma	
Catnb+/lox(ex3);WAP-Cre	Stabilizing deletion of Exon 3 (codons 5 to 80) of β-catenin	Squamous metaplasia	[93]
Catnb+/lox(ex3);MMTV-Cre	Luminal expression		
MMTV-ΔN90-β-catenin x $p53^{+/-}$	Stabilizing deletion of β-catenin on a p53 heterozygous background	Adenocarcinomas	[175]
	Luminal expression	Metastases	
K5-ΔN57-β-catenin	Stabilizing deletion of first 57 amino acids of β-catenin	Precocious development during pregnancy	[38,74]
	Basal expression	Hyperbranching	
		Multiparous mice develop invasive basal-type carcinoma	
MMTV-Wnt1	Wnt1 expression in luminal epithelia	Expansion of $K14^{+}$, $cd24^{+}$, $cd29/49f^{hi}$ basal stem cells	[33,67,84,85]
		Hyperbranching	
		Adenocarcinoma	
MMTV-Wnt10b	Expression of long isoform of Wnt10b in luminal epithelia	Precocious alveologenesis	[61]
		Hyperbranching	
		Adenocarcinoma	
MMTV-Lrp6	Expression of Lrp6 in luminal epithelia	Hyperplasia no tumors	[88]
$Apc^{Min/+}$	Truncating mutation of APC at codon 850	Focal alveolar hyperplasias	[178]
		Mammary carcinomas (7 to 20% depending on strain background)	
$Apc^{+/1572}$	Truncating mutation of APC at codon 1572	Multifocal mammary adenocarcinomas (85.7%)	[95]
		Pulmonary metastases	
K14-cre-$Apc^{580/+}$	Truncating mutation of APC at codon 580	Mammary adenocarcinomas	[94]
	Basal expression		
BLG-Cre; $Apc^{580S/580S}$	Truncating mutation of APC at codon 580	Delayed ductal morphogenesis	[179]
	Luminal expression	Metaplasia	
MMTV-rtTA;TRE2-myc-tagged Axin-(IRES) GFP	Doxycycline-inducible Axin in luminal epithelia	Loss of alveologenesis	[76]
WAP-β-engrailed	β-engrailed expressed luminally	Failure to maintain alveoli	[77]
MMTV-β-engrailed		Lactation defect	
$Lrp5^{-/-}$	Loss of Lrp5	Loss of placodes	[34,39]
		Mild ductal hypoplasia	
		Reduced regenerative capacity	
		Resistant to Wnt-induced tumorigenesis	
$Lrp6^{-/-}$	Loss of Lrp6	Impaired sprouting, placode and fat pad development	[40]
		Decreased number of TEBs in heterozygotes	
$Wnt4^{-/-}$	Loss of Wnt4	Delayed side-branching	[69]
$Wnt5a^{-/-}$	Loss of Wnt5a	Accelerated ductal morphogenesis	[64]
K5-rtTA/tetO-DKK1	Ectopic expression of Dkk1	Loss of mammary line and all placodal development	[36]
K14-Cre/Pygo2 flox	Loss of Pygo2 in mammary epithelium	Loss and small placodes	[38]
		Progressive loss of progenitor cell population	
$Pin1^{-/-}$	Loss of Pin1	Severe decrease in ductal and alveolar development during pregnancy	[80]
$RANK^{-/-}$	Loss of RANK receptor	Loss of alveologenesis	[78,79]
		Failure to lactate	
		Impaired side-branching in transplanted null outgrowths	
$Lef1^{-/-}$	Loss of Lef1	Impaired placodes	[37]
		Impaired bud morphogenesis	
$Rspo^{-/-}$	Loss of Rspo1	Defective side branching	[73]

APC, adenomatous polyposis coli; DKK, Dickkopf; GFP, green fluorescence protein; LRP, low-density lipoprotein-related protein; MMTV, mouse mammary tumor virus; Pygo2, pygopus homolog 2; RANK, receptor activator of NF-κB; TEB, terminal end bud; WAP, whey acidic protein.

signaling remain silent, but weak Conductin-lacZ expression is seen in basal cells and stroma surrounding the terminal end buds [23]. Conductin/Axin2 is an endogenous target gene that is faithfully expressed in response to canonical Wnt signaling and serves as a negative feedback loop to downregulate the pathway [63]. These observations indicate that β-catenin signaling occurs at low levels. Genetically reducing canonical Wnt receptors produces mild effects: $Lrp5^{-/-}$ mutants and $Lrp6^{+/-}$ mutants show fewer terminal end buds, $Lrp5^{-/-}$ mutants show delayed ductal extension, and $Lrp6^{+/-}$ mutants show reduced ductal branching. Compound $Lrp6^{+/-}$;$Lrp5^{-/-}$ mutants show no ductal outgrowth, however, demonstrating a requirement for canonical Wnt pathway activity [34,39,40].

Current evidence suggests that noncanonical Wnt antagonism of canonical Wnt pathways is important. For example, loss of Wnt5a, which stimulates noncanonical signaling pathways during other polarized morphogenic events, increases cytoplasmic and nuclear β-catenin and accelerates ductal outgrowth [64]. Importantly, Wnt5a is an essential mediator of TGFβ, suggesting that low thresholds of β-catenin signaling are maintained during pubertal ductal morphogenesis through TGFβ and Wnt5a antagonism.

Links between progesterone and β-catenin signaling during ductal maturation

A significant body of data shows that progesterone not only promotes Wnt expression but also induces competence to respond to β-catenin signaling within specific mammary progenitors. Progesterone receptor (PR) is expressed in most luminal cells in juveniles but becomes downregulated and interspersed with PR-negative cells in adults. This patterning event requires PR and generates PR-negative progenitors that produce side branches and alveoli during pregnancy [65]. Expression of a stabilized form of β-catenin lacking N-terminal phosphorylation degron sequences (MMTV-ΔN89β-catenin) uniformly within the luminal epithelium induces signaling within this progenitor population, resulting in precocious development at regular intervals along ducts [66,67]. In the absence of PR, however, ΔN89β is only able to produce this effect at ductal tips [66,67]. This observation suggests that PR specifies and spatially patterns alveolar progenitors by inducing a factor that is essential for β-catenin's nuclear entry or transcriptional activity and thus renders cells poised to receive a β-catenin signal [66,67]. Progesterone also induces several paracrine factors – for example, Wnt4 and receptor activator of NF-κB ligand (RANKL) – in PR-positive luminal cells that signal to their neighbors [68-70]. A recent study has highlighted the importance of these paracrine effectors during estrus, showing that their expression correlated with β-catenin target gene expression in both luminal and basal cells and with increased stem cell activity [71].

Canonical Wnt signaling during pregnancy

Wnts and RANKL also function downstream of progesterone to stimulate side-branching and alveolar development during pregnancy [68-70]. Wnt4, Wnt5b and Wnt6 peak sequentially – and deletion of the earliest, Wnt4, delays side-branching, whereas ectopic Wnt4 expression promotes side-branching [58,69,72]. The gene for Rspo1 – which encodes an alternative (non-Wnt) ligand for Lrp6, and activates β-catenin signaling – is expressed during early pregnancy, and its deletion similarly affects side-branching [73]. Canonical Wnt signaling exerts dose-dependent effects on ductal branching: $Conductin^{lacZ/+}$ mice show no phenotype but further pathway de-repression in $Conductin^{lacZ/lacZ}$ mice results in mild hyperbranching and strong activation in K5-ΔN57β-catenin or MMTV-Wnt1 mice produces dramatic hyperbranching and gross ductal distortion [23,67,74,75]. Importantly, canonical Wnt signaling in $Conductin^{LacZ/LacZ}$ mice and in MMTV-Wnt1;$Conductin^{LacZ/+}$ mice is detected in basal cells [67,69]. This observation suggests that Wnts expressed by luminal cells regulate ductal morphogenesis in a paracrine manner by inducing a canonical Wnt signaling response in basal cells [67,69].

In sharp contrast, a series of studies have shown that activation of β-signaling within specific hormone receptor-negative luminal cell populations promotes and is required for alveologenesis. Expression of MMTV-ΔN89β-catenin within luminal cells induces precocious alveologenesis in virgin mice [67,75]. β-Catenin signaling reporters are transiently expressed during normal alveologenesis, and inhibiting endogenous β-catenin signaling within luminal cells by expressing a negative regulator (Axin) or a dominant negative form of β-catenin (β-engrailed) prevents alveologenesis and caused alveolar degeneration [36,50,76,77]. Together, these studies establish that luminal β-catenin signaling is essential for alveologenesis and survival.

The upstream activator remains to be defined but is unlikely to involve Wnts since luminal cells express no obvious Wnt receptor and alveologenesis proceeds in the absence of Lrp5 [34]. Intriguingly, RANKL stabilizes β-catenin in other contexts and, like β-catenin, specifically promotes the proliferation of PR-negative cells [78,79]. $RANKL^{-/-}$ mice and mice expressing Axin show similar impairment in alveologenesis [76,79]. Importantly, Pin1, which provides a Wnt-independent route to β-catenin stabilization, is critical for alveolar development during pregnancy [24,80]. Downstream targets of β-catenin signaling remain obscure. C-*myc* and cyclin D_1 are reduced and increased in mice where the pathway is suppressed and is increased, respectively [67,75-77].

Cyclin D_1, however, is dispensable for the proliferative response to MMTV-ΔN89β-catenin, MMTV-Wnt1 and RANKL, and instead drives early and later postpartum waves of cell division [79,81,82].

In summary, Wnts and RANKL promote stem cell amplification [33,71] and branching [58,69,72] by paracrine induction of β-catenin signaling in basal cells [34,39,40,67,71]. In contrast, β-catenin signaling in luminal progenitors is essential for alveologenesis and is probably induced by Wnt-independent mechanisms (for a model, see Figure 3) [67,76,77].

Deregulating β-catenin signaling in mammary stem and progenitor cells induces cancer

Just as regulation of stem cells is key to understanding the function of β-catenin signaling during embryonic and adult mammary development, its proto-oncogenic properties may be viewed as arising from unbridled stem and early progenitor expansion. More than 25 years ago, retroviral insertion resulting in Wnt1 activation was found to induce mammary tumors – and, subsequently, transgenic expression of Wnt1, Wnt3 and Wnt10b was shown to produce similar effects [61,83,84]. A series of studies have revealed that exposure of glands to excessive Wnt1 or activated ΔN89β-catenin leads to progressive accumulation of cells displaying stem and progenitor marker profiles and enhanced colony-forming ability and bipotency *in vitro* [67,85-88]. Reporter studies indicate that Wnt1 activates β-catenin signaling in a paracrine manner, increasing basally located multipotent stem cells at the expense of differentiated myoepithelial cells [67]. The pivotal role of a canonical Wnt pathway versus a noncanonical Wnt pathway in this process has been demonstrated by the fact that $Lrp5^{-/-}$ mice are highly resistant to Wnt1 tumor development and show significantly delayed tumor onset [39,40]. Collectively, these studies indicate that paracrine signals from MMTV-expressing luminal cells promote β-catenin signaling within a basal stem cell population and induce mixed lineage tumors. In keeping with this scenario, continuous Wnt1 expression is required for tumor maintenance [89].

Mice expressing activated β-catenin from the MMTV promoter also develop adenocarcinoma with 100% penetrance [75,90]. In this mouse model, however, reporter studies have shown that β-catenin signaling occurs within a distinct subset of luminal cells scattered along ducts with alveolar progenitor marker profiles and functional characteristics [67]. These studies show that deregulation of β-catenin signaling within luminal progenitor cells also produces tumors of mixed lineage [67,85-88]. Significantly, in humans, basal-type hormone receptor-negative breast cancer is thought to arise from luminal progenitors. Target genes include matrix metalloproteinases, cyclin D_1 and c-*myc*, which are upregulated in both MMTV-Wnt1 and MMTV-ΔN89β-catenin glands. Cyclin D_1, however, is dispensable for tumor initiation [81,91]. In fact, MMTV-ΔN89β-catenin mice lacking cyclin D_1 present accelerated tumor onset and higher tumor burden [91]. Lastly, canonical Wnt pathway activity has been implicated in mediating radiation resistance in mammary Sca1-positive progenitors by inducing Survivin expression [92].

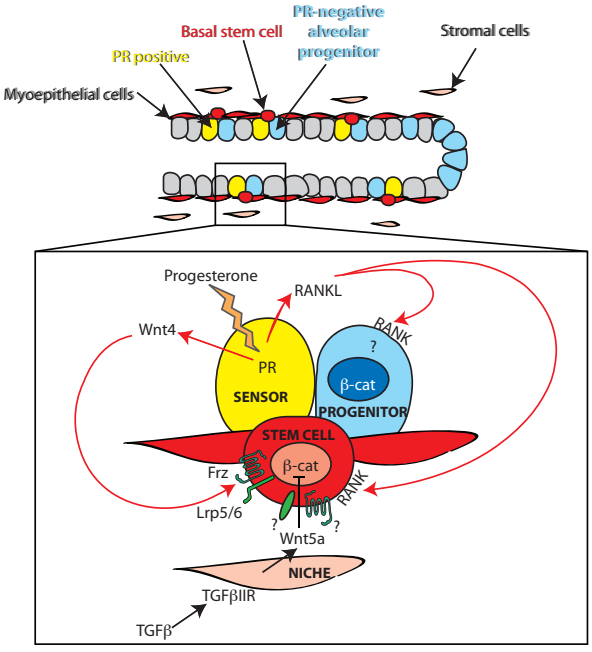

Figure 3. Hypothetical model for paracrine activation of β-catenin signaling in the mammary gland. Progesterone receptor (PR) induces Wnts and receptor activator of NF-κB ligand (RANKL). These paracrine factors bind to their respective receptors low-density lipoprotein-related protein (LRP)5/6 and RANK in basal cells. β-catenin signaling in this cell type induces stem cell amplification and branching. Transforming growth factor beta (TGFβ) signaling within stromal cells restrains ductal morphogenesis by inducing Wnt5a. Wnt5a antagonizes canonical Wnt signaling, possibly via noncanonical Wnt pathways. β-Catenin signaling in luminal progenitors is essential for alveologenesis. As luminal cells lack Wnt receptors, however, it is likely that β-catenin signaling is induced in this cell-type by Wnt-independent mechanisms.

The response to β-catenin signaling is highly context dependent. K5-ΔN57β-catenin mice develop basal preneoplasia and invasive ductal carcinomas, while activation of β-catenin by MMTV-cre-targeted or whey acidic protein-cre-targeted exon 3 deletion shows squamous metaplasia [74,93]. Likewise, a mutant APC generated mammary adenocarcinomas when expressed in the basal layer but induced squamous metaplasia when expressed in differentiating luminal cells [94]. APC mutants have also revealed tissue-specific dose responses. The $APC^{Min/+}$ mutation, which results in high levels of β-catenin, predominantly causes intestinal polyposis, while APC mutants that generate intermediate levels of β-catenin

preferentially induce mammary tumors [95]. The strain background influences the tumorigenic properties of $APC^{Min/+}$ and genetic studies have begun to identify tissue-specific modifier loci [96].

Tumor microenvironmental factors also exert powerful effects on the tumor phenotype. For example, inhibiting TGFβ signaling in stromal cells by expression of a dominant negative receptor (metallothionine inducible dominant-negative TGFβ receptor II transgene) increases β-catenin signaling within the epithelium of MMTV-Neu-induced and MMTV-PyVmT-induced tumors. This is accompanied by a remarkable change in tumor phenotype from one that is principally luminal to one with mixed luminal and basal cell types [97]. Similar effects are produced by eliminating the TGFβ target gene and the paracrine effector, Wnt5a [97]. The tumor microenvironment is in turn influenced by Wnt1, which impairs laminin deposition, disrupts cell polarity, promotes stromal hyperplasia and induces hedgehog signaling activity, suggesting important connections between these pathways in tumor onset [67,98].

β-catenin in human breast cancer

Studies in mice strongly suggest that deregulated β-catenin signaling increases the breast cancer risk by inducing stem and early progenitor cell accumulation [67,85]. Consistent with this hypothesis, a gene expression signature derived from MMTV-Wnt1 tumor-initiating cells was found to have prognostic value in determining poor outcome associated with specific breast cancer types, including basal-like and hormone receptor-negative cancers [99]. Genes of the canonical Wnt pathway featured prominently in a breast-to-lung metastasis signature that was significantly correlated with poor prognosis and reduced overall survival. Finally, Wnt1 tumors share transcriptional patterns with $BRCA1^{+/-}$ tumors and human basaloid tumors, and several studies have linked Lrp6 and nuclear β-catenin with triple-negative breast cancer [40,100-103].

Stabilizing mutations in the β-catenin N-terminal sequence found in other human cancers are uncommon in breast cancer and, until recently, were only reported in benign fibromas [104,105]. Such mutations have now been found in 25% of metaplastic breast cancers [106]. In contrast to the rarity of mutation, β-catenin expression and/or localization are often abnormal in human breast cancer [5,24,106-109]. Increased cytoplasmic and nuclear β-catenin levels have been documented in 40% of primary breast cancers and correlated with poor prognosis and worse patient survival [24,107,109-114]. These observations suggest that upstream elements of Wnt and/or other pathways that stabilize β-catenin catenin are deregulated in breast cancer. Indeed, a significant number of breast tumors develop in an environment of unopposed Wnt ligand activity [112,115-120]. Inactivation of genes encoding secreted Wnt pathway inhibitors, such as secreted frizzled-related protein (sFRP), Wnt inhibitory factor and DKK, occurs frequently in breast cancer. sFRP1 levels are diminished in infiltrating (or invasive) ductal carcinoma, and gain and loss of sFRP1 suppresses and promotes invasiveness of breast cancer, respectively [112,116,117,119,120]. sFRP1, sFRP5, Wnt inhibitory factor-1 and DKK3 are transcriptionally silenced by promoter methylation in 27 to 100% of breast cancers [118,121-125]. Silencing of sFRP1, in particular, is a prognostic indicator of poor overall survival. One study found DKK1 to be preferentially expressed in hormone receptor-negative cancers; as DKK is a Wnt target gene, however, this elevation may actually indicate augmented pathway activity [126].

There is also mounting evidence for excess Wnt ligand production, aberrant receptor activation and loss of noncanonical Wnt antagonism in human breast cancer. Wnt2, Wnt4 and Wnt10b mRNA upregulation has been reported [112,127-134]. Wnt5a, which negatively regulates β-catenin activity during mammary development, is lost in a proportion of human infiltrating (or invasive) ductal carcinoma and is a powerful predictor of recurrence [135]. FRZ1 and FRZ2 upregulation occurs and a splicing mutation in LRP5 that induced β-catenin activity was found in 85% of tumors [136,137]. Two studies have reported increased LRP6 expression in human breast cancer, particularly in triple-negative basal-like subtypes [40,102]. One study showed that Mesd, an inhibitor of Lrp5/6 folding, suppressed growth of MMTV-Wnt1 tumors despite the presence of excess ligand and without adverse effects on normal Wnt-driven processes, suggesting that Lrp6 may have potential as a therapeutic target [102].

Disheveled, which promotes β-catenin signaling, is upregulated in up to 50% of primary breast cancers [113,138]. Moreover, although mutations in negative regulatory components such as APC and Axin, are rare, loss of heterozygosity, downregulation or epigenetic silencing of these genes occurs in up to 70% of breast tumor cell lines and cancers [105,108,109,114,139-152]. Nuclear transcriptional cofactors such as Pygo2 show upregulated expression in a number of human invasive ductal cancers and are required for anchorage-independent growth in human breast cancer cell lines [153]. EZH2, which promotes nuclear translocation of β-catenin and transcriptional activity, induces ductal hyperplasia and involution defects in MMTV-EZH2 mice and is highly expressed in human breast carcinomas [154].

A number of Wnt target genes are implicated in breast cancer. Genes encoding c-*myc* and cyclin D_1 are amplified and upregulated in 40% of human breast cancers [107,155-158]. Cyclin D_1 is dispensable for Wnt or

β-catenin murine tumorigenesis; however, Myc activates Wnt signaling by suppressing DKK and sFRP1, providing positive feedback to sustain the cancerous state [81,91, 159]. Other relevant β-catenin targets include WISP1, Slug, Twist, Sox17, and Limb Heart Bud [100,160-163]. Sox17, a transcriptional antagonist of canonical Wnt signaling, is epigenetically silenced in 74.3% of breast tumors [162]. Limb Heart Bud, an important embryonic patterning gene, is upregulated in MMTV-Wnt tumors and many human breast cancers. This gene is highly expressed in 50% of basal breast cancers, again suggesting that this breast cancer subtype may involve reactivation of dormant developmental signaling pathways [163].

An increasing body of evidence suggests that stabilization of β-catenin by Wnt-independent routes, such as Pin1, p53, PTEN/Akt and NF-κB pathways, plays a significant role in breast cancer. For example, Pin1 upregulation correlates with increasing cancer grade (25% in ductal carcinoma *in situ*, 71.4% in grade II tumors, and 89.5% in grade III tumors) [164], and is associated with increased expression of β-catenin in breast tumors [24]. Components of the NF-κB pathway, which can differentially regulate β-catenin, are also activated in estrogen receptor-negative/ErbB2-positive breast cancers (86%) [165].

Loss of PTEN and p53 tumor suppressors has also been linked to induction of β-catenin in breast cancer. Germline PTEN mutations are common in patients with Cowden's syndrome, showing a lifetime risk of 25 to 50% for developing breast cancer, and sporadic PTEN loss correlates with invasive breast carcinomas (48%, n = 151) [166,167]. Significantly, overexpression of PTEN partially inhibits Wnt-induced tumorigenesis; conversely, loss of PTEN and activation of Akt caused an expansion of normal and malignant breast stem cells via activation of β-catenin [26,167,168]. These data suggest that the ability of PTEN to act as a tumor suppressor is mediated partly through the Wnt pathway. In addition to PTEN, inhibitor of differentiation binding-1, Slit-2 and melanoma differentiation association gene-7 proteins all regulate β-catenin in breast cancer cells through the phosphatidylinositol-3-kinase pathway [169-171].

Mutations in the p53 gene cause the heritable disease Li–Fraumeni syndrome, which is characterized by an 18-fold enhancement in susceptibility to breast cancer. p53 mutation occurs in 50% of all human cancers and in 20% of sporadic breast cancer (for a review see [172]). β-Catenin is downregulated by p53 and is also degraded through a p53-dependent mechanism, which included Siah1, SIP and Ebi [27-29]. Adding to the complexity, Siah1 has splice variants that differentially affect β-catenin in breast cancer cells [173,174]. MMTV-ΔNβ-catenin tumors induced in $p53^{+/-}$ mice are more malignant and metastatic than those in $p53^{+/+}$ mice [175]. Likewise, patients with breast cancers displaying high levels of β-catenin and p53 had worse survival [176]. Additionally, p63 was shown to be highly upregulated in metaplastic breast carcinomas (86.7%, n = 15) [177].

Conclusions

Evidence from murine models show that deregulated β-catenin signaling in basal stem cells or luminal progenitors induces breast tumors. This event correlates with disturbances in the homeostatic balance between stem cell and early progenitor cell self-renewal and the production of differentiated progeny. There are numerous pathways, in addition to the canonical Wnt pathway, which regulate β-catenin. Mutations in β-catenin are rare but deregulated β-catenin signaling occurs frequently in human breast cancer and may be particularly relevant for basal-type and triple-negative subtypes, suggesting important links between reactivation of developmental pathways and this breast cancer subtype.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

Abl, Abelson tyrosine kinase; APC, adenomatous polyposis coli; DKK, Dickkopf; EBI, EBV-induced G-protein coupled receptor 1; ErbB, erythroblastic leukemia viral oncogene; EZH2, enhancer of zeste homolog 2; FGF, fibroblast growth factor; GSK3β, glycogen synthase kinase-3β; IKK, IκB kinase; LRP, low-density lipoprotein-related protein; K5, keratin 5; MMTV, mouse mammary tumor virus; Muc1, mucin 1; NF, nuclear factor; PR, progesterone receptor; PTEN, phosphatase and tensin homolog; PTHrP, parathyroid hormone-related peptide; Pygo2, pygopus homolog 2; RANKL, receptor activator of NF-κB ligand; sFRP, secreted frizzled-related protein; SIAH1, seven in absentia homolog 1; SIP, Siah interacting protein; TGFβ, transforming growth factor beta; WISP, Wnt-inducible signaling pathway protein.

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