Gap Junctions and Wnt Signaling in the Mammary Gland: A Cross-talk?

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1. Abstract

Connexins (Cxs), the building blocks of gap junctions (GJs), exhibit spatiotemporal patterns of expression and regulate the development and differentiation of the mammary gland, acting via channel-dependent and channel-independent mechanisms. Impaired Cx expression and localization are reported in breast cancer, suggesting a tumor suppressive role for Cxs. The signaling events that mediate the role of GJs in the development and tumorigenesis of the mammary gland remain poorly identified. The Wnt pathways, encompassing the canonical or the Wnt/β-catenin pathway and the noncanonical β-catenin-independent pathway, also play important roles in those processes. Indeed, aberrant Wnt signaling is associated with breast cancer. Despite the coincident roles of Cxs and Wnt pathways, the cross-talk in the breast tissue is poorly defined, although this is reported in a number of other tissues. Our previous studies revealed a channel-independent role for Cx43 in inducing differentiation or suppressing tumorigenesis of mammary epithelial cells by acting as a negative regulator of the Wnt/β-catenin pathway. Here, we provide a brief overview of mammary gland development, with emphasis on the role of Cxs in development and tumorigenesis of this tissue. We also discuss the role of Wnt signaling in similar contexts, and review the literature illustrating interplay between Cxs and Wnt pathways.

Keywords: Mammary Gland; Breast Cancer; Gap Junctions; Connexins; Wnt Pathways

2. Introduction

The mammary gland continues to develop postnatally and is considered a valuable model for studying epithelial physiology and pathology. In addition to the role of soluble mediators as systemic regulators of breast tissue development and differentiation, the local microenvironment has emerged as a major regulator almost two decades ago [1-7]. Disruption of the mammary epithelial microenvironment is linked to breast cancer development [8-10]. Neighboring cells with which mammary epithelial cells directly interact to establish homocellular and heterocellular junctions have gained considerable interest, both in developmental and tumorigenesis contexts [11, 12]. Gap junctions (GJs) regulate the development and differentiation of the mammary gland. Altered expression and localization of their building blocks, connexin (Cx) proteins, are reported in breast cancer, making them candidate tumor suppressors [13-28]. Indeed, a tight spatiotemporal regulation governs the expression of Cxs in the mammary gland throughout its development [29-34]. Studies addressing the role of Cxs in mammary epithelial differentiation or tumorigenesis implicate channel-independent mechanisms for Cxs beyond their classical GJ-dependent roles [35, 36, 24]. However, the downstream pathways through which Cxs act remain elusive. Both branches of Wnt signaling, the canonical or the Wnt/β-catenin pathway and the noncanonical pathway, execute key roles in mammary gland development and differentiation, and altered Wnt signaling is associated with breast cancer [37-48]. The involvement of Cxs and Wnt pathways in similar processes suggests a cross-talk in the breast tissue. Induction of Wnt1 expression in a mammary epithelial cell line enhances Cx43 expression and gap junctional intercellular communication (GJIC) [49]. Similarly, stimulation of mammary cocultures with Wnt3a upregulates the expression of Cx43 [50]. Overexpression of Wnt5a in the mammary epithelium impairs lactation in mice by altering Cx43 function [51]. Although Cxs are downstream targets of Wnt signaling in the mammary epithelium, the interplay between the two is poorly investigated and is not defined in terms of the biological context, possibly due to the scarcity of studies. In support of a cross-talk, we have demonstrated negative regulation of the Wnt/β-catenin pathway by Cxs, as a mechanism to induce differentiation [36] or to suppress tumorigenesis [24] in the mammary epithelium. Furthermore, our recent findings indicate a role for Cxs in regulating the noncanonical Wnt pathway in the breast tissue (unpublished

data). In this review, we elaborate on the roles of Cxs and Wnt pathways in mammary development and breast cancer. We next discuss the cross-talk between Cxs and Wnt signaling in nonbreast tissues, and we propose a model for their interaction in the mammary gland in developmental and tumorigenic contexts.

Cxs may act as upstream negative regulators or as downstream positive effectors of Wnt/β-catenin signaling, depending on the biological context. The "positive effector" role of Cxs is linked to developmental and pathological processes, such as ovarian folliculogenesis and endometrioid adenocarcinomas [52, 53]. This role is additionally defined in the context of cardiac differentiation and function, whereby induction of Cx expression downstream of canonical Wnt signaling enhances spontaneous beat rate and improves cardiac conduction [54, 55]. Cxs act as "negative regulators" of the Wnt/β-catenin pathway as a mechanism to regulate cell cycle entry in kidney cells [56]. Furthermore, reconstitution of Cx expression reverses the malignancy of glioma and colon cancer cells by inhibiting canonical Wnt signaling [57, 58]. In light of the above findings, we propose a model in which a similar cross-talk exists between Cxs and Wnt signaling in the mammary gland. Whether Cxs play the role of an "upstream negative regulator" or a "downstream positive effector", this is likely governed by the context. During developmental stages, canonical Wnt signaling induces the expression of Cxs that execute channel-dependent and channel-independent functions to regulate the morphogenesis and differentiation of the mammary tissue, and subsequently act as inhibitors of canonical Wnt signaling to maintain homeostasis and suppress tumorigenesis. The downregulation of Cx expression in early stages of breast cancer leads to the loss of this control and induces hyperproliferation into a primary tumor. In the context of advanced-stage breast cancer, aberrant canonical Wnt signaling upregulates Cx expression to support collective migration and drive tumor metastasis.

3. Development of the Mammary Gland

Extensive remodeling governs the development of the mammary gland and predominates it during adulthood. The anatomical and molecular events that accompany the development of the mammary gland from prenatal stages to weaning post lactation are well characterized [59, 60]. Murine models have been mainly used for studying the development of the mammary gland. In brief, development commences during

embryogenesis, and is initiated by the formation of bilateral milk lines, or mammary ridges, which develop into mammary placodes and then into epithelial bulbs that invade the underlying mesenchyme. Bud elongation produces a mammary sprout that further invades the fat pad precursor mesenchyme. A rudimentary ductal system develops within the mammary adipose tissue upon lumen formation and branching of the sprout, and continues with the isometric growth until the neonatal phase. Subsequently, the mammary gland remains quiescent until puberty [59, 61, 62]. At puberty, estrogen mediates the formation of terminal end buds (TEBs) at the tips of the branching ducts. TEBs direct elongation and branching of the ductal tree, characterized by epithelial proliferation and migration, and regress upon reaching the edges of the fat pad [59, 63-65]. Further side branching occurs with each estrous cycle in response to progesterone [66]. During pregnancy, progesterone and prolactin stimulate the development of alveolar buds at the ends of the branching ducts. At this point, epithelial cells within alveoli undergo structural and functional differentiation [59, 67, 68]. At parturition, reduced progesterone levels and sustained production of prolactin induces milk secretion in alveoli. Upon cessation of lactation, epithelial apoptosis results in involution of the mammary gland and regression into a prepregnancy state [59, 68].

In humans, the mature female breast encompasses lobules, milk ducts, connective tissue and adipose tissue. Terminal duct lobular units (TDLUs), the functional units of the breast, consist of a terminal duct that connects to the ductal system and leads to a lobule, a cluster of glandular milk-secreting structures termed alveoli or acini. Luminal epithelial cells line alveoli (lobular epithelium) and ducts (ductal epithelium), and are surrounded by a discontinuous layer of myoepithelial cells. A basement membrane supports the mammary epithelium and forms contacts with both luminal epithelial and myoepithelial cells in TDLUs. The stroma consists of an extracellular matrix (ECM) and stromal cells (fibroblasts, adipocytes, endothelial cells and immune cells) which underlie the basement membrane [69].

Development of the mammary gland is tightly regulated by systemic (endocrine) and local factors (microenvironment) that act together to ensure the proper spatiotemporal regulation of proliferation, differentiation and apoptosis, thereby preventing developmental defects and neoplastic transformation [70]. Stromal cells are part of the local factors that play important roles in orchestrating morphogenetic events in

the developing mammary gland. Fibroblasts, for instance, trigger epithelial branching morphogenesis in a 3-dimensional (3-D) fibroblast-epithelial coculture model [4]. Macrophages or eosinophils are also required for mouse TEB formation and ductal branching, which are impaired in mice lacking those cells in their mammary glands [2]. Furthermore, mice dually treated with estradiol and progesterone to induce alveologenesis have reduced ability to form alveolar buds upon depletion of macrophages [1]. Macrophages also regulate mammary gland involution, whereby the execution of epithelial apoptosis, alveolar regression and adipocyte repopulation fails in macrophage-devoid mice [5].

In addition to stromal cells, the role of ECM signaling in regulating mammary gland development is extensively documented [3, 7, 6]. Interactions of the epithelial and myoepithelial compartments with the underlying ECM generate biochemical and mechanical signals that dictate normal mammary architecture and function [71]. Thus, disruption of cell-ECM interactions is associated with developmental defects and breast tumorigenesis. Conditional deletion of β1-integrin, a major ECM receptor, from the basal compartment of mouse mammary epithelium alters ductal branching pattern and impairs lobuloalveolar development [6]. The ECM is dynamically deposited and degraded throughout the developmental stages of the mammary gland, further supporting its role in mammary morphogenesis. Indeed, ECM components and remodeling enzymes undergo spatial and temporal expression in the developing mammary glands of mice [72, 3, 7, 73]. Therefore, normal morphogenesis of the mammary gland is not only contingent upon tight hormonal regulation, but is also dependent on the presence of a well-regulated microenvironment.

4. Connexins in Mammary Gland Development

Cxs are expressed in most cell types and exhibit evolutionary conservation among chordates [74]. Twenty Cx genes have been identified in mice and 21 in humans. Most Cx genes share a similar structure consisting of two exons separated by one intron. The first exon is untranslated, while the second contains the coding region and the 3'-untranslated region (3'-UTR) [75]. Cx proteins consist of highly conserved cytoplasmic N-terminal domain, two extracellular loops with four transmembrane domains, and variable intracellular loop and cytoplasmic C-terminal domain that account for functional differences among Cx isoforms [76-78].

Cx43 is the most abundantly and ubiquitously expressed Cx protein, making it the most studied Cx isoform [76-78]. Cxs oligomerize to form hexameric structures referred to as hemichannels or connexons, and docking of two connexons in adjacent cell membranes forms a GJ channel. Oligomerization of identical Cxs forms homomeric connexons, while heteromeric connexons result upon association of different Cx isoforms. In addition, homotypic or heterotypic GJ channels result from docking of identical or different connexons, respectively. Structures formed upon accumulation of thousands of GJ channels at the membrane are referred to as GJ plaques or GJs [76-78]. GJs connect the cytoplasms of two adjacent cells, allowing intercellular exchange of ions, second messengers (Ca^{2+}, cAMP and IP3) and metabolites (sugars, amino acids and small peptides) less than 1.5 kDa in size [76-78]. In addition to their classical channel-dependent roles, Cxs execute channel-independent functions by associating with signaling molecules, enzymes, cytoskeletal and junctional proteins, among others [76, 77]. The expression and turnover of Cxs are tightly regulated. The loss of this regulation, whether in the form of loss of expression, mutations or altered GJIC, is associated with disease outcomes, including cancer [79-81].

The expression patterns of Cxs in the mammary gland are spatiotemporally defined. In mouse models, luminal epithelial cells express Cx26, Cx30 and Cx32, while the expression of Cx43 is limited to the mammary myoepithelium [33]. In contrast, the expression of Cx43 is evident in both epithelial cell layers in reduction mammoplasties of normal women, with luminal epithelial cells expressing additionally Cx26 [82, 83]. Despite a well-characterized spatial expression of Cxs in the human mammary gland, temporal expression patterns remain elusive, and are linked to sampling limitations and inability to obtain normal breast tissue samples at the various developmental stages of the mammary gland. Majority of studies investigating the temporal expression of Cxs utilized mouse models [29, 32, 30, 31, 33, 34, 84, 85].

Cxs play important roles in normal development and physiology of the mammary gland. Cx26 and Cx43 knockout mice die *in utero* and at birth, respectively, making it impossible to study the role of Cx26 and Cx43 in mammary glands of these mice [86, 87]. Autosomal dominant Cx43 mutation ($Cx43^{I130T/+}$) delays ductal elongation and reduces gland size relative to body weight in prepubertal mice. Although milk production and ejection are not affected, mutant mice have impaired mammary epithelial proliferation,

leading to reduced gland size at parturition [88]. In a similar model ($Cx43^{G60S/+}$), mammary gland development is delayed in virgin mice. Ductal elongation, branching, TEB formation and relative mammary gland weight are reduced, but the morphology of the mammary gland at parturition is not affected [21]. Furthermore, milk secretion and *ex vivo* oxytocin-induced milk ejection into the ducts are impaired [21, 22]. Indeed, knocking down Cx43 or blocking GJIC in primary mammary organoids of wild-type mice inhibits myoepithelial contractility in response to oxytocin stimulation [27]. Replacement of Cx43 with Cx32 in a heterozygous knock-in mouse model ($Cx43^{Cx32/+}$) reduces postnatal growth and survival of pups. This is attributed to defects in milk ejection but not in mammary gland development or milk production [89]. Heterozygous $Cx43^{Cx26/+}$ mutation has similar effects on pup survival and growth, does not affect milk production, but is associated with reduced branching of ductuli, number and size of secretory alveoli in lactating mice [90]. In a Cx26 conditional knockdown mouse model, where the physiological surge in mammary Cx26 that accompanies pregnancy and lactation is inhibited, normal development and function of the mammary gland are retained, indicating that basal levels of Cx26 are sufficient [25]. Interestingly, transgenic mice overexpressing Cx26 under the control of keratin 5 promoter (K5-Cx26), which exhibits constitutive activity in myoepithelial cells, are unable to feed their pups despite normal mammary gland development and milk production. In fact, *ex vivo* oxytocin stimulation of mammary organoids isolated from transgenic mice fails to induce contraction, and ectopic expression of Cx26 in myoepithelial cells alters the expression of endogenous Cx43, leading to disrupted GJIC [27]. This illustrates the importance of spatial regulation of Cx expression in normal functioning of the mammary gland. Conditional inactivation of Cx26 gene in the mammary epithelial compartment ($Cx26^{fl/fl}$ x MMTV-Cre) affects mouse mammary glands in a stage-dependent manner. The loss of Cx26 before puberty does not alter ductal elongation or branching, but it impairs lobuloalveolar development and function during pregnancy and lactation, respectively. These effects are due to increased apoptosis and are not associated with reduced mammary epithelial proliferation. In contrast, the loss of Cx26 during later stages of pregnancy does not affect mammary development or function, illustrating the temporal effects of Cx expression in the mammary gland [91]. Indeed, Cx26 acts downstream of prolactin signaling in the mammary epithelium during early pregnancy. Mouse mammary

epithelial transplants devoid of prolactin receptor form alveolar buds that fail to undergo lobuloalveolar development during pregnancy. This is concomitant with reduced expression of Cx26, suggesting a role in prolactin-induced mammary development [92]. The spatiotemporal expression patterns of murine mammary Cxs and the developmental defects associated with their altered expression are summarized in Table 1 [93, 27, 91, 21, 22, 88-90].

Table 1. The spatiotemporal expression patterns of murine mammary Cxs and the developmental abnormalities in mouse models of altered Cx expression.

Cx Isoform	Cell Compartment	Developmental Stage	Mouse Model	Developmental Abnormality	References
Cx26	Luminal epithelium	Pregnancy Parturition Lactation	K5-Cx26: Ectopic expression of Cx26 in myoepithelial cells	Impaired milk ejection	[27]
			$Cx26^{fl/fl}$ x MMTV-Cre: Conditional deletion of Cx26 gene in mammary epithelial cells before puberty	Impaired lobuloalveolar development and lactation	[91]
			$Cx43^{Cx26/+}$: (see below)		[90]
Cx30	Luminal epithelium	Pregnancy Parturition Lactation			
Cx32	Luminal epithelium	Parturition Lactation	$Cx43^{Cx32/+}$: (see below)		[89]
Cx43	Myoepithelium	Pregnancy Parturition Lactation	$Cx43^{G60S/+}$: Autosomal dominant point mutation (G60S) in one Cx43 allele	Delayed ductal elongation, branching and TEB formation Reduced gland size Defective milk secretion and ejection	[21, 22]
			$Cx43^{I130T/+}$: Autosomal dominant point mutation (I130T) in one Cx43 allele	Delayed ductal elongation Reduced gland size	[88]
			$Cx43^{Cx32/+}$: Replacement of one Cx43 allele with Cx32	Impaired milk ejection	[89]
			$Cx43^{Cx26/+}$: Replacement of one Cx43 allele with	Reduced ductular branching Reduced alveolar	[90]

			Cx26	number and size	

We have previously demonstrated channel-dependent and channel-independent roles for Cx43 in differentiation of the mammary gland [35, 36]. Blocking GJIC in CID-9 mouse mammary cell strain under differentiation-permissive conditions (in the presence of exogenous basement membrane) downregulates the expression of β-casein, a milk protein and a differentiation marker. Furthermore, induction of GJIC in the absence of a basement membrane is sufficient to induce mammary epithelial differentiation [35]. Indeed, these effects are independent of ECM-induced signal transducer and activator of transcription 5 (STAT5) [94]. Subsequently, we illustrated involvement of GJ complex assembly (Cx43, among other Cxs, α-catenin, β-catenin and ZO-2) in differentiation of mouse mammary epithelial SCp2 cells. The role of GJ complex assembly in mammary epithelial differentiation is partly mediated by the recruitment of β-catenin to the membrane, thereby preventing its nuclear translocation, which induces the expression of proliferation and cell cycle genes [36].

5. Connexins in Breast Tumorigenesis

Aberrant patterns of Cx expression and localization are linked to breast cancer. Reduced Cx43 expression is reported in human breast cancer tissues at various stages of tumor progression, in carcinogen-induced rat mammary tumors and in breast cancer cell lines [14]. In addition to impaired expression, progressive alteration of Cx43 localization is found in human mammary dysplasia and breast cancer tissues, as compared to normal breast tissues. Cx43 exhibits intercellular punctate localization in normal breast tissues and diffuse cytoplasmic pattern in breast cancer tissues, indicating loss of GJIC [17]. Indeed, a positive correlation is established between Cx43 levels and improved disease outcome in breast cancer patients, and Cx43 is proposed as an independent prognostic marker [95]. In addition to the dysregulation of Cx43, reduced or complete loss of Cx26 expression is reported in breast cancer cell lines, compared to nontumorigenic human mammary epithelial cells, conferring a potential role to Cx26 in tumor suppression [15].

The tumor suppressive roles of Cxs in the mammary gland are supported by both *in vitro* and *in vivo* studies. We have previously demonstrated a tumor suppressive role for Cx43 in the breast. Overexpression of Cx43

in MCF-7 and MDA-MB-231 cells, human breast cancer cell lines, reduces proliferation, cell cycle progression and invasiveness and reverses their characteristic malignant phenotype. These effects are independent of GJIC, given that overexpression of a C-terminus-truncated version of Cx43 fails to restore the wild-type Cx43 phenotype. Furthermore, blocking GJIC in Cx43-overexpressing cells does not reverse the effects of Cx43, corroborating the involvement of channel-independent mechanisms [24]. Likewise, overexpression of Cx26 in MCF-7 and MDA-MB-435 cells reduces proliferation, anchorage-independent growth, migration and invasion [18, 20]. The effects of Cx26 on MDA-MB-435 cells are channel independent, as shown by the expression of a GJIC-incompetent Cx26 form that phenocopies the effects of wild-type Cx26 [20]. Overexpression of Cx26 or Cx43 in MDA-MB-231 and MDA-MB-435 cells suppresses xenograft tumor growth in nude mice [13, 16]. Furthermore, migration of MDA-MB-231 cells is impaired upon exposure to Cx43-rich biovesicles extracted from plasma membranes of donor cells overexpressing functional Cx43-based GJs and capable of forming GJs with cells [28]. Conditional mammary gland-specific knockout of Cx26 in mice predisposes the mammary gland to primary tumors in DMBA-induced breast cancer model [26]. Similarly, mice with heterozygous Cx43 mutation show higher susceptibility to mammary tumor lung metastasis following DMBA treatment [23]. *In vitro*, silencing Cx43 in Hs578T cells, human breast cancer cell line, enhances proliferation and anchorage-independent growth. This is associated with the upregulation of vascular endothelial growth factor (VEGF), a proangiogenic molecule, and downregulation of thrombospondin 1 (TSP-1), an antiangiogenic molecule [19]. We have recently shown that silencing Cx43 in nontumorigenic human mammary epithelial cell line, HMT-3522 S1 cells, enhances proliferation and cell cycle progression, and induces mislocalization of membranous β-catenin (unpublished data). In addition, Cx43-silenced cells display morphogenetic defects typical of breast cancer initiation. These include loss of apical polarity, misorientation of the mitotic spindle, multilayering and loss of lumen, thus indicating disruption of normal acinar morphology (Bazzoun et al; submitted).

Collectively, the above studies illustrate key roles of Cxs in development and tumorigenesis of the mammary gland. The involvement of channel-independent mechanisms in Cx signaling suggests a link between Cxs and cellular pathways that execute overlapping roles with those of Cxs in the mammary gland.

The developmental pathways which mediate Cx signaling in the mammary gland are yet to be investigated. Evidence supports interplay between Cxs and Wnt signaling in nonbreast tissues and in a multitude of biological contexts. In the mammary epithelium, canonical and noncanonical Wnt signaling regulate the expression and function of Cx43 [49-51]. In addition, our earlier findings indicate that the Wnt/β-catenin pathway is a modulator of Cx signaling in differentiation [36] and tumorigenesis [24] of mammary epithelial cells. This suggests that the Wnt pathways are potential candidates for relaying Cx signals within the mammary gland in development and cancer.

6. Connexins as Regulators of Wnt Signaling

A. Connexins in Canonical Wnt Signaling

i. Canonical Wnt Pathway

The Wnt/β-catenin pathway (or the canonical Wnt pathway) is one of the three best characterized Wnt pathways, which also include the planar cell polarity (PCP) and the Wnt/calcium pathways. The Wnt/β-catenin pathway is involved in β-catenin-mediated regulation of developmental gene expression, essential for embryogenesis and adult tissue homeostasis. Deregulation of this pathway is associated with developmental defects and adult diseases, including cancer [96-98].

In the absence of a Wnt ligand, two scaffolding proteins, adenomatous polyposis coli (APC) and Axin as well as casein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK-3) form a complex in the cytoplasm, referred to as the β-catenin destruction complex. CK1 and GSK-3, serine/threonine protein kinases, sequentially phosphorylate β-catenin on specific N-terminal amino acid residues (on serine 45, and subsequently on threonine 41, serine 37 and serine 33, respectively). This marks β-catenin for ubiquitination (dually phosphorylated β-catenin on serine 33 and 37 is recognized by β-TrCP, E3 ubiquitin ligase) and subsequent proteasomal degradation leading to a reduction in the cytoplasmic pool of β-catenin available for nuclear translocation. Consequently, Wnt/β-catenin target genes are repressed by the DNA-bound T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors. TCF/LEF acts as transcriptional repressor by forming a complex with Groucho (Gro)/transducin-like enhancer (TLE), which interacts with histone deacetylases (HDACs) to mediate histone deacetylation and chromatin compaction [96-98].

In the presence of Wnt, the ligand binds to its receptor and coreceptor, Frizzled (Fzd) and low-density lipoprotein receptor-related protein 5 or 6 (LRP5/6), respectively. This complex (Wnt-Fzd-LRP5/6) triggers Fzd-mediated recruitment of Dishevelled (Dvl), a scaffolding protein, which in turn recruits Axin along with its associated GSK-3 and CK1 to the membrane, resulting in phosphorylation of LRP5/6 by GSK-3 and CK1. Phosphorylation leads to the activation of LRP5/6, which recruits the Axin-GSK-3-CK1 complex, thereby amplifying phosphorylation of LRP5/6 and enhancing the recruitment of the Axin complex as well. As a result, the β-catenin destruction complex (APC-Axin-CK1-GSK-3) is disrupted. This stabilizes β-catenin and leads to its accumulation and translocation to the nucleus, where it acts as a transcriptional coactivator. In the nucleus, β-catenin displaces Gro/TLE to form a complex with TCF/LEF, thereby converting the latter into a transcriptional activator and inducing the expression of genes involved in cell cycle progression, including c-Myc, cyclin-dependent kinase 1 (CDK1) and cyclin D1 (Fig. 1). Wnt/β-catenin target genes also include components of the Wnt/β-catenin pathway itself that may act as agonists or antagonists, conferring self-regulatory properties to the pathway [98, 96, 97].

ii. Role of Canonical Wnt Pathway in Mammary Gland Development

The role of Wnt signaling in mammary gland development and breast tumorigenesis is well documented [99-101]. The earliest detectable event marking the activation of Wnt signaling during mammary development is the expression of Wnt10b in the mammary line and Wnt6 in the surface ectoderm of mouse embryos at embryonic day E11.25 [61]. Wnt signaling components are expressed in a cell type-specific and stage-dependent manner in the developing mammary gland [102, 103]. The expression patterns of Wnt ligands are summarized in Table 2 [104, 61, 105-110].

Table 2. The spatiotemporal expression patterns of Wnt ligands in the murine mammary gland.

Developmental Stage	Cell Compartment	Wnt Ligand	References
Embryonic	Mammary line	Wnt5a Wnt6 Wnt10b	[61, 105]
	Mammary placode	Wnt1 Wnt2 Wnt3 Wnt5a Wnt6 Wnt7b	[61, 105]

		Wnt10a Wnt10b Wnt11	
	Mammary bud	Wnt1 Wnt2 Wnt3 Wnt3a Wnt4 Wnt5a Wnt5b Wnt7b Wnt10b Wnt11	[105]
Pubertal	TEB	Wnt2 Wnt4 Wnt5a Wnt5b Wnt6 Wnt7b	[106]
	Duct	Wnt4 Wnt5b Wnt6	[106]
Adult	Luminal epithelium	Wnt4 Wnt5a Wnt5b Wnt7b	[104, 109, 110]
	Myoepithelium	Wnt5a Wnt5b Wnt10a	[109, 110]
Pregnancy		Wnt2 (early, mid) Wnt4 (early, mid) Wnt5a (early, mid) Wnt5b (early, mid, late) Wnt6 (early, mid, late) Wnt7b (early) Wnt10b (early)	[107, 108]

Canonical Wnt signaling initiates mammary gland morphogenesis in mouse embryos. Activation of canonical Wnt signaling in the mammary region overlaps with the onset of mammary morphogenesis and localizes to mammary placodes and buds thereafter. Forced activation of canonical Wnt signaling using Wnt3a accelerates placode formation in cultured embryos. Conversely, ectopic expression of the Wnt inhibitor Dikkopf 1 (DKK1) in the surface epithelium of transgenic embryos blocks placode development [105]. Formation of rudimentary mammary buds is inhibited in mouse embryos with homozygous LEF-1 mutation [37], while homozygous mutation in LRP5 reduces the size of mammary placodes in mouse

embryos and alters ductal elongation and TEB numbers in virgin mice [111]. Similarly, LRP6 knockout mouse embryos have smaller mammary placodes and fat pad and lack ductal branching, whereas heterozygous LRP6 mutation alters TEB numbers and ductal branching in juvenile and adult mice, respectively [45]. Canonical Wnt signaling also mediates progesterone-induced side branching in mammary ducts. Ectopic expression of Wnt1 rescues side branching of ducts in mammary epithelial transplants of mice with homozygous mutation in progesterone receptor, indicating that the canonical Wnt signaling acts downstream of progesterone. This latter induces the expression of Wnt4, and mammary bud implants derived from Wnt4-deficient mouse embryos show impaired ductal branching during early pregnancy [112]. Expression of a constitutively active form of β-catenin causes precocious lobuloalveolar development and differentiation in mouse mammary glands. Indeed, virgin mammary glands of these transgenic mice resemble those of wild-type pregnant mice in terms of development and functional differentiation, show lobular hyperplasia during pregnancy and regress into a midpregnant state, characteristic of virgin transgenic mice, post lactation. The transgenic mice develop multiple aggressive adenocarcinomas early on during their lifetime [41].

iii. Role of Canonical Wnt Pathway in Breast Tumorigenesis

In addition to regulating development and differentiation of the mammary gland, aberrant Wnt/β-catenin signaling plays a role in breast cancer. Reduced levels of membranous β-catenin and enhanced nuclear activity are linked to poor disease outcome in breast cancer patients and are proposed as independent prognostic factors [40, 113]. β-catenin mutations at phosphorylation sites that target it for ubiquitination and subsequent degradation, as well as inactivating APC mutations, lead to stabilization of β-catenin and constitutive activation of the Wnt/β-catenin signaling. Although nuclear and cytoplasmic accumulation of β-catenin are reported in breast cancer, APC and β-catenin mutations, commonly associated with other cancers, are absent or rare and restricted to benign and metaplastic breast tumors [114-120]. This suggests that deregulated Wnt/β-catenin signaling in breast cancer is not a consequence of mutations in components of this pathway. In support of this, defective expression, localization or epigenetic patterns of canonical Wnt components are associated with breast cancer. Wnt ligands, receptors and coreceptors are overexpressed in

breast cancer [121, 122, 42, 123, 124]. For instance, expression of FZD1 and FZD2 receptors is upregulated in breast cancer tissues [42]. Similarly, LRP6 is overexpressed in breast cancer cell lines and tissues and is required for activation of canonical Wnt signaling, cell proliferation and xenograft tumor growth, while administration of LRP6 antagonist *in vivo* prevents the growth of MMTV-Wnt1 tumors [124]. Interestingly, expression of an aberrantly spliced internally truncated form of LRP5 coreceptor is found in breast cancer tissues. This form is essential for β-catenin stability and activity, cell proliferation and tumor growth in a xenograft mouse model [123]. Altered expression and epigenetic regulation of other components in the Wnt/β-catenin pathway are also common. Amplification and upregulation of Dvl1, a scaffolding protein that recruits the β-catenin destruction complex, are reported in primary breast tumors [125]. APC promoter hypermethylation and reduced expression are detected in breast cancer tissues and correlate with active Wnt/β-catenin signaling [44, 126]. Epigenetic silencing and promoter hypermethylation of Wnt antagonist genes, including secreted frizzled-related protein (sFRP) family, Wnt inhibitory factor 1 (WIF1) and DKK, are present in breast cancer cell lines and in primary breast tumors [127, 128]. Reduced sFRP expression accounts for activation of canonical Wnt signaling, and expression of sFRP suppresses proliferation of breast cancer cells [129, 128].

Although Cxs and Wnt/β-catenin signaling play overlapping roles in the mammary gland, scarce evidence supports a link between these pathways in the breast tissue [49, 50]. We have previously shown Cx channel-independent signaling as an upstream negative regulator of the Wnt/β-catenin pathway in the breast. Cx43 associates with β-catenin at the membrane and inhibits its nuclear translocation, as a mechanism to induce differentiation [36] or to suppress tumorigenesis [24] in mammary epithelial cells. The interplay between Cxs and canonical Wnt signaling exists in a number of other tissues, where Cxs act as upstream negative regulators or as downstream positive effectors of the Wnt/β-catenin pathway.

iv. Cross-talk between Connexins and Canonical Wnt Signaling

Connexins as Upstream Negative Regulators of Canonical Wnt Signaling

Evidence supports negative regulation of the Wnt/β-catenin pathway by Cx signaling in cardiac, bone, kidney, nervous and colon tissues [54, 56-58, 130-132].

Overexpression of Cx43 in lithium-stimulated neonatal rat cardiomyocytes (lithium mimics Wnt signaling by inhibiting GSK-3β) reduces β-catenin transcriptional activity. Association and colocalization of Cx43 and β-catenin at the membrane suggests that Cx43 inhibits canonical Wnt signaling via β-catenin sequestration [54].

The knockout of Cx43 or Cx37 in osteocytes results in the accumulation of β-catenin and increased expression of Wnt/β-catenin target genes. These effects are associated with enhanced Wnt/β-catenin-dependent processes, including osteogenic response to mechanical loading and resistance to fractures in bones [130, 132]. Interestingly, pannexin 3 (Panx3), a member of a recently identified family of GJ proteins, also inhibits Wnt/β-catenin signaling in bones. Overexpression of Panx3 in osteoprogenitor cells cultured under proliferation conditions reduces proliferation and induces cell cycle arrest. Panx3 exerts its effects by enhancing the activity of GSK-3β, leading to the phosphorylation of β-catenin and the reduction of its cytoplasmic levels. This is coupled to a decrease in β-catenin nuclear localization and activity. As a result, levels of cyclin D1 and phosphorylated retinoblastoma (Rb), involved in G1 to S phase progression, are reduced [133].

In a study on the role of adhesion molecules in cell proliferation, Cx43 synergizes the effects of N-cadherin in suppressing β-catenin/TCF transcriptional activity, as a mechanism to upregulate p21 and reduce proliferation and cell cycle progression in HEK293 human embryonic kidney cells. Notably, the effects of Cx43 are channel dependent [56].

Reconstitution of Cx43 in glioma stem cells (GSCs) impairs tumorsphere formation and proliferation. In addition, increased expression of glial fibrillary acidic protein (GFAP), an astrocytic differentiation marker, and reduced expression of CD133, a stem cell marker, are noted, indicating differentiation and impaired self-renewal capacity. Overexpression of Cx43 is also associated with reduced invasiveness *in vitro*, and xenografts of Cx43-transduced GSCs exhibit smaller tumor size, reduced proliferation and better differentiation, compared to their mock counterparts, suggesting that Cx43 inhibits tumorigenicity of GSCs. Notably, overexpression of Cx43 in GSCs does not restore GJIC, indicating that the observed effects of Cx43 are due to channel-independent mechanisms. Microarray analysis revealed reduced expression of

Wnt/β-catenin target genes, including stemness-related genes (Nanog, Oct4 and Sox2), in Cx43-transduced GSCs. Furthermore, overexpression of Cx43 induces the expression of E-cadherin, and knocking down E-cadherin in Cx43-transduced GSCs is sufficient to restore invasiveness, indicating that Cx43 negatively regulates the Wnt/β-catenin pathway in GSCs via an E-cadherin-dependent mechanism [58]. The loss of Cx43, but not GJIC, is associated with differentiation of human neural progenitor cells as a consequence of enhanced canonical Wnt signaling. Silencing Cx43 triggers neurogenesis by increasing the protein levels and transcriptional activity of β-catenin, thereby upregulating the expression of proneuronal genes [131].

Ectopic expression of Cx43 in HT29 colon cancer cell line reduces anchorage-dependent, anchorage-independent and xenograft growth. Notably, ectopically expressed Cx43 localizes mainly to intracellular vesicular compartments and fails to form GJs, suggesting the implication of channel-independent mechanisms in tumor suppression. In addition, Cx43 associates with β-catenin and reduces TCF transcriptional activity in HT29 cells, indicating negative regulation of the Wnt/β-catenin signaling, a mechanism through which Cx43 could exert its tumor suppressive effects [57].

While the above studies described Cxs as negative regulators of canonical Wnt signaling, others reported positive regulation of Cxs downstream of the Wnt/β-catenin pathway. This illustrates possible existence of a negative feedback mechanism, whereby Cxs act as both downstream targets and inhibitors of the Wnt/β-catenin pathway.

Connexins as Downstream Positive Targets of Canonical Wnt Signaling

In cardiac and skeletal muscle cells, the Wnt/β-catenin pathway upregulates the expression of Cxs, mainly Cx43, and GJIC [54, 134-136, 55]. GJIC and Cx43 expression are enhanced in neonatal rat cardiomyocytes and skeletal myoblasts in response to lithium-stimulated activation of canonical Wnt signaling, and are associated with increased spontaneous beat rate in cardiomyocytes [54, 135]. Indeed, activation of the Wnt/β-catenin signaling acts downstream of cyclic strain to upregulate Cx43 expression in mouse embryonic stem cells, thereby inducing cardiac differentiation [136]. Canonical Wnt signaling also mediates the effects of β1-integrin on Cx mRNA expression (Cx40, Cx43 and Cx45) in mouse embryonic stem cell-derived cardiomyocytes at advanced stages of differentiation [134]. Furthermore, inhibition of β-catenin or

GSK-3α/β in HL-1 cells, mouse cardiomyocyte cell line, prevents mesenchymal stem cell (MSC)-induced upregulation of Cx43 and improvement in cardiac conduction, suggesting that MSCs alleviate cardiac arrhythmias via activation of the canonical Wnt signaling [55].

A similar pattern of Cx and GJ regulation is reported in Xenopus embryos, ovarian follicles and ovarian carcinomas, umbilical vein endothelial cells and retinal pigment epithelial cells [137, 53, 138, 52, 139, 140].

Studies summarized above clearly illustrate interplay between Cxs, mainly Cx43, and the Wnt/β-catenin pathway in several tissues, with the former acting either as downstream targets (positive effectors) or as upstream negative regulators of Wnt signaling. Whether Cxs play the downstream role of a "positive effector" or are upstream "negative regulator" of Wnt signaling, the interplay between the two is context specific. Studies defining Cxs as downstream targets (positive effectors) for the Wnt/β-catenin pathway correlate tissue development and differentiation-driving events to effective GJ communication. As previously stated, induction of Cx43 expression, among other cardiac Cxs (Cx40 and Cx45), downstream of canonical Wnt signaling is associated with the acquisition of cardiac differentiation and function [54, 134, 136, 55]. The "positive effector" role of Cxs is additionally associated with developmental processes, such as embryogenesis, angiogenesis and ovarian folliculogenesis [137, 138, 52, 139]. On the other hand, this role is evident in the context of disease pathogenesis, including ovarian cancer [53] and proliferative vitreoretinopathy [140]. In contrast to acting as downstream targets in developmental contexts, the inhibitory effects of Cxs upstream (i.e. "negative regulator") of the Wnt/β-catenin pathway are associated with differentiation or tumor suppression as most studies indicate [54, 56, 58, 57]. Hence, Cxs likely undergo a switch in role from a "positive effector" into a "negative regulator" of the Wnt/β-catenin pathway upon establishment of tissue development to suppress tumorigenesis. During growth and differentiation-driving events of the normal mammary gland, we speculate positive regulation of Cxs downstream of active canonical Wnt signaling to induce Cx-mediated morphogenesis and differentiation [21, 22, 27, 35, 137, 138, 52, 139, 54, 134, 136, 55]. Within the same context, hyperactive Wnt/β-catenin signaling impairs mammary development [50]. In the context of a differentiated mammary tissue, however, Cxs act to suppress the Wnt/β-catenin pathway in order to maintain homeostasis and to execute tumor suppressive effects (Fig. 2a)

[36, 56-58, 54]. In early stages of breast cancer, the loss of Cx expression triggers the formation of primary tumor by activating canonical Wnt signaling [76, 141, 142, 24], whereas in the context of advanced breast cancer-driving events, aberrant Wnt/β-catenin signaling induces Cx expression to support collective migration and tumor metastasis (Fig. 2b) [53, 140].

B. Connexins in Noncanonical Wnt Signaling

i. Noncanonical Wnt Pathway

The noncanonical Wnt signaling is a branch of Wnt signaling that encompasses multiple β-catenin-independent pathways and regulates embryogenesis and adult tissue homeostasis. As such, aberrant noncanonical Wnt signaling is associated with developmental defects and adult diseases, particularly cancer [143-148].

Noncanonical Wnt signaling regulates epithelial apicobasal polarity (asymmetry along the apical-basal axis within a cell), PCP (the coordinated organization of cells within a tissue plane, also referred to as tissue polarity), cell junctions, mitotic spindle orientation, actin cytoskeletal dynamics and cell migration. Noncanonical Wnt pathways are triggered by specific family members of Wnt ligands that signal through Fzd receptors, like the canonical branch, but use alternatives to LRP5/6 where coreceptors are involved. Owing to the ligand and coreceptor differences, the noncanonical Wnt pathways regulate signaling cascades different from that underlying canonical Wnt signaling downstream of Dvl recruitment to the ligand-receptor-coreceptor complex. In addition, while the activation of the canonical Wnt pathway regulates gene expression, noncanonical Wnt signaling is also associated with nontranscriptional outcomes. The PCP and the Wnt/calcium pathways are by far the best characterized among the noncanonical Wnt pathways [143-149].

The PCP pathway activates Ras homolog (Rho) GTPases, namely Rac and Rho, and c-Jun N-terminal kinase (JNK), which induce cytoskeletal rearrangements [143, 145]. The PCP pathway is activated when a noncanonical Wnt ligand binds to Fzd and its coreceptor (ROR2, RYK, PTK7 or NRH1). Dvl is subsequently recruited and associates with Dishevelled-associated activator of morphogenesis 1 (Daam1),

which activates Rho via a guanine nucleotide exchange factor (GEF). Rho in turn activates Rho-associated kinase (ROCK), a major regulator of the actin cytoskeleton. Daam1, on the other hand, mediates binding of profilin to actin. In addition, Dvl mediates activation of Rac, which activates JNK. Profilin, ROCK and JNK induce actin cytoskeletal reorganization [143, 145, 147]. The PCP pathway is known to regulate actin polymerization, as a mechanism to control cell morphology and polarized cell migration [143]. Microtubules constitute another cytoskeletal element regulated by the PCP pathway, which orients the mitotic spindle relative to cell-cell contacts or to an embryo symmetry axis [144] (Fig. 3a). Due to its role in cell division orientation and directional cell movement, the PCP pathway regulates morphogenetic processes, such as gastrulation, neurulation and organ morphogenesis [145, 147].

The Wnt/calcium pathway, on the other hand, activates Fzd-associated heterotrimeric G proteins besides Dvl and regulates intracellular calcium levels by stimulating or inhibiting calcium release from the endoplasmic reticulum (ER). One consequence of calcium release is the activation of the Rho GTPase Cdc42 through protein kinase C (PKC). Another important outcome is the activation of calcium/calmodulin-dependent protein kinase II (CaMKII), which in turn activates nuclear factor of activated T-cells (NFAT), a transcription factor [143, 149] (Fig. 3b). The Wnt/calcium pathway regulates several aspects of embryogenesis, such as ventral cell fate, tissue separation and convergent extension, and is thought of as a modulator of PCP signaling [143].

Fzd-independent pathways are identified as components of noncanonical Wnt signaling, although less characterized than the PCP and the Wnt/calcium pathways [150, 151]. The Fzd coreceptors ROR2 and RYK harbor functional extracellular Wnt-binding domains and can act as Wnt receptors independently from Fzd activation [150] (Fig. 3c). ROR2 and RYK regulate developmental processes in several tissues and are associated with cell polarity, migration and asymmetric cell division [150-153].

Due to a cross-talk among noncanonical Wnt pathways, these pathways are alternatively considered as one signaling network with diverse biological outcomes. Studies modeling the noncanonical Wnt pathways as such highlighted the roles of Rho GTPases as important downstream effectors of noncanonical Wnt

signaling. RhoA, Rac1 and Cdc42 are known to regulate cytoskeletal dynamics involving the microtubule and actin networks, thereby controlling mitotic spindle orientation, cell shape changes, motility and invasion. Rho GTPase signaling also regulates polarity, intercellular junctions and cell-ECM interactions, hence the implication of the deregulation of Rho GTPases in mammary gland tumorigenesis [154-157, 148, 147].

ii. Role of Rho GTPases in Mammary Gland Development

Rho GTPase signaling components are implicated in various stages of mammary gland development, from embryogenesis to involution, and their aberrant expression and/or activity contributes to breast tumorigenesis [158, 159].

Inhibition of Rac1 or ROCK, a downstream effector of RhoA, in an organoid culture of mammary tissue blocks duct initiation and disrupts branching pattern, respectively, indicating a role for Rac1 and RhoA in morphogenesis of the mammary gland [43]. Expression of a dominant-negative form of Rac1 or its downstream effector p21-activated kinase 1 (PAK1) enhances the contractility of mouse myoepithelial cells *in vitro*. Consistent with these observations, the expression of a constitutively active form of Rac1 or a catalytically active form of PAK1 induces myoepithelial relaxation, demonstrating a role for Rac1 signaling in controlling the contraction/relaxation cycle of myoepithelial cells and thus in lactation [46]. Conditional deletion of Rac1 in mouse mammary glands delays involution via STAT3-dependent mechanism [48].

A study on a 3-D culture of primary mammary epithelial cells isolated from Cdc42 conditional knockout mice unveiled a role for Cdc42 in morphogenesis of the mammary gland. Cdc42 deficiency reduces cell proliferation and survival and alters the number and size of acini, concomitant with disruption of acinar morphology. Furthermore, apicobasal polarity, mitotic spindle orientation and lumen formation, which represent key morphogenetic features of normal mammary epithelium, are disrupted [160]. Paradoxically, normal morphogenesis of the mammary gland is also disrupted in a tetracycline-regulatable Cdc42 overexpression mouse model. This suggests the importance of tight regulation of Cdc42 levels for normal mammary gland development. Cdc42-overexpressing mammary glands exhibit TEB hyperbudding and

trifurcation, ductal tree hyperbranching and altered epithelial-stromal interactions, which are known to regulate branching. Consistent with these observations, primary mammary epithelial cells isolated from Cdc42-overexpressing mammary glands form dysmorphic invasive acini in 3-D cultures, coupled to enhanced expression of ECM proteins and remodeling enzymes in their stromal counterparts. Interestingly, the phenotypic abnormalities observed upon Cdc42 overexpression are not a consequence of enhanced cell proliferation or survival, nor are they associated with disruptions in apicobasal polarity or mitotic spindle orientation. They are rather due to enhanced epithelial contractility and migration [47]. Taken together, gain-of-function and loss-of-function studies clearly illustrate redundancy in Cdc42 effects, suggesting that its role in mammary gland morphogenesis is highly contingent upon a tight balance of its levels, and perhaps activity. In addition to regulating the morphogenesis of the mammary gland, Cdc42 plays a role in its proper functioning. Conditional knockout mice lacking Cdc42 in mammary alveolar epithelial cells during lactation inadequately nourish their pups, leading to stunted growth. This is attributed to impaired alveologenesis as a consequence of disrupted apical-basal polarity and cell-cell adhesion, which result in premature exfoliation of the alveolar epithelium [161].

iii. Role of Rho GTPases in Breast Tumorigenesis

Rho GTPases are overexpressed or hyperactivated in human breast tumors [39, 38, 158]. In addition, the expression of Rho GTPase regulators and effectors is altered in breast cancer tissues [158, 162-164]. A link is established between Rho GTPase expression levels and cell motility and invasion *in vitro*. Cdc42 and Rac regulate the formation of filopodia and lamellipodia, respectively, at the leading edge of a motile cell, while Rho regulates the formation of stress fibers and actomyosin contractility at the rear end [156]. The presence of a cross-talk among Rho GTPases during cell motility is also reported. For instance, Forster resonance energy transfer (FRET) biosensor imaging revealed a biphasic localization of RhoA activity at the leading edge of epidermal growth factor (EGF)-stimulated MTLn3 rat mammary adenocarcinoma cells. This spatiotemporal pattern of RhoA activity is critical for coordinating the functions of Rac1 and Cdc42 during the formation of protrusions [165]. Primary mammary epithelial cells from Cdc42-overexpressing mammary glands display enhanced contractility and migration. Specifically, Cdc42 overexpression upregulates ECM

proteins and remodeling enzyme levels in stromal cells, and disrupts epithelial-stromal interactions, further supporting a role for Cdc42 in breast cancer invasion [47]. Consistent with those findings, the knockdown of Cdc42 in MTLn3 cells impairs EGF-induced protrusion, barbed end formation and F-actin accumulation at the protruding edges, which are concomitant with reduced motility, suggesting a role for Cdc42 in breast cancer cell motility [166]. siRNA-mediated silencing of RhoA or RhoC impairs invasiveness of MDA-MB-231 cells [167]. Interestingly, ROCK mediates the invasion of amoeboid breast cancer cells through matrix metalloproteinase (MMP)-independent mechanism, by regulating myosin light chain (MLC) organization and the generation of forces that cause deformation of the underlying collagen fibers, thereby allowing cells to invade the ECM [168]. Silencing RhoC in MTLn3 cells impairs protrusion formation and directionality in response to EGF stimulation [169]. In addition, RhoC-depleted MTLn3 cells exhibit altered morphology and function of the ECM-degrading invadopodial protrusions and reduced invasive potential [170]. Rac1 counteracts the activity of RhoC in MTLn3 cells by inducing the disassembly of invadopodia. Considering the role of Rac1 in the formation of lamellipodia, this effect is believed to sustain the proper balance between matrix-degrading and locomotory protrusions for optimal cell invasion [171]. In fact, knocking down Rac1 induces membrane ruffling and impairs motility in EGF-stimulated MTLn3 cells. This is due to altered formation of focal adhesions at the leading edge, rendering the protrusions unstable [172].

In addition to their role in breast cancer invasion, Rho GTPases alter the morphogenesis of mammary epithelial tissue, an event that marks breast cancer initiation, both *in vitro* and *in vivo* [47]. Indeed, Rho GTPase signaling plays a role in regulating morphogenetic aspects of mammary epithelial cells, including cell-cell adhesion, cell-ECM interactions, apicobasal polarity, mitotic spindle orientation and lumen formation [160, 47, 161]. Rho GTPases also mediate preneoplastic transformation, tumor growth, angiogenesis and metastasis in breast cancer. Ectopic expression of RhoA leads to immortalization of primary human mammary epithelial cells [173]. In contrast, silencing RhoA reduces the proliferation of MDA-MB-231 cells and suppresses xenograft tumor growth, angiogenesis and lung metastasis in mice [167, 174]. Similarly, inhibiting Rac1 in MDA-MB-435 cells impairs tumor growth, angiogenesis and metastasis in a nude mouse model [175].

iv. Cross-talk between Connexins and Rho GTPase Signaling

As previously mentioned, intercellular adhesion and communication, which are key aspects of a differentiated mammary epithelium, are disrupted in breast cancer. Rho GTPase activities are spatiotemporally regulated to control the establishment and maintenance of epithelial apicobasal polarity and cell-cell junctions, particularly tight junctions (TJs) and adherens junctions (AJs) [154, 157, 176]. FRET biosensor studies showed spatiotemporal localization patterns of RhoA, Rac1 and Cdc42 activities along the apical and lateral membrane domains of Madin-Darby canine kidney (MDCK) epithelial cells during cystogenesis. Specifically, Rac1 activity at the lateral membrane exceeds that at the apical membrane during late cystogenesis, and induction of Rac1 activity at the apical membrane of mature cysts disrupts apical-basolateral polarity, TJs and mitotic spindle orientation [177]. Spatiotemporal Rac1 activity is also implicated in the establishment of AJs. FRET biosensor imaging showed that local Rac1 activation is induced upon the formation of nascent AJs, leading to junction stabilization in endothelial cells [178]. RhoA colocalizes with AJs in the developing mouse brain, and conditional knockout of RhoA in neural progenitor cells of the forebrain and midbrain disrupts AJs, suggesting a role for RhoA in maintenance of AJs [179]. RhoA also regulates the maintenance of both apicobasal polarity and TJ localization in retinal progenitor cells during vertebrate embryonic development [180]. Similarly, Cdc42 regulates the establishment of cell polarity and junction assembly in a mammalian model of early embryonic development. Cdc42-null embryoid bodies show homogenous cortical distribution of F-actin and lack the characteristic distribution of the microtubule-organizing center (MTOC) and Golgi complex, indicating absence of cell polarity. In addition punctate cell-cell contacts containing TJ and AJ markers are formed, and continuous TJ or AJ belts fail to assemble [181].

Rho GTPase signaling is also known to regulate GJ function and assembly. Blocking the activities of Rho family proteins by overexpressing the guanine nucleotide dissociation inhibitor (GDI) Rho GDIα under the control of the cardiac-specific α-myosin heavy chain (α-MHC) promoter reduces the expression levels of Cx40 in mouse hearts and is associated with conduction defects [182]. In a similar study where C3-exoenzyme expression is utilized, inhibition of Rho GTPase activities in mouse lenses reduces Cx50

expression levels [183]. Consistent with those findings, calpeptin-stimulated RhoA activity in HL-1 cells, mouse cardiac myocyte cell line, upregulates the expression levels of Cx43 [184]. In parallel, Rho GTPases also affect Cx localization. For instance, Cx43 localization is altered in response to Rac1 inhibition in neonatal rat cardiac myocytes [185]. Likewise, Cx26 and Cx32 are mislocalized in hepatocytes of Cdc42-deficient mouse livers [186]. Rho GTPases further regulate GJs at the level of assembly and permeability. Inhibiting Rho activity in primary rabbit corneal epithelial cells by C3-exoenzyme microinjection impairs the assembly of Cx43-based GJs [187]. In addition, C3-exoenzyme-induced inhibition of RhoA reduces GJIC in rat cardiac myocytes [188]. Notably, other families of GTPases, mainly the Ras family, are also implicated in the regulation of Cx expression levels, GJ formation and GJIC [189-197].

In contrast to above, others demonstrated that Cxs are upstream regulators of Rho GTPase signaling. Cx43 activates the RhoA-ROCK pathway, as a mechanism for bradykinin-induced vascular contraction [198]. Furthermore, a role for Cx43 in Rac1 activation and actin cytoskeletal reorganization is proposed in breast cancer cells [199]. Blocking GJIC induces phosphorylation of Cdc42 in mouse ventricular zone precursors, resulting in its inactivation [200]. Unlike the aforementioned studies that reported positive regulation of Rho GTPases by Cxs, one study demonstrated enhanced Rac1 and RhoA activities in 3T3 mouse embryonic fibroblasts in response to Cx43 knockdown. This is followed by enhanced migration and actin cytoskeletal reorganization [201]. The variable effect of Cxs on Rho GTPases suggests that Cxs regulate Rho GTPase signaling in a cell type-specific and/or context-dependent manner. Cxs also regulate other GTPases, such as Rap1. In WEHI-231 cells, murine B lymphoma cell line, Cx43 mediates B-cell receptor (BCR)-, integrin (LFA-1)- and chemokine (CXCL12)-induced Rap1 activation and the subsequent spreading and adhesion of B cells to vascular endothelial cells [202, 203]. Cx43 further regulates BCR- and integrin-induced B cell motility, in addition to chemokine-stimulated directed and transendothelial migration downstream of Rap1 activation [202].

Although a cross-talk between Cxs and Rho GTPases is implied, the literature describing such a link remains scarce, and almost no evidence supports its existence in the breast tissue. In one study however, the noncanonical ligand Wnt5a is proposed to impair lactation in mice through regulating Cx functions. In

contrast to wild-type mice, overexpression of Wnt5a in the mammary epithelium inhibits oxytocin-induced milk ejection and sustains the phosphorylation of Cx43 after parturition [51]. Studies summarized above suggest positive regulation of Cx expression and function downstream of Rho GTPase signaling in tissue morphogenesis, differentiation and pathology [182-184]. Considering the dual roles of Cxs and Rho GTPases in development and tumorigenesis of the mammary gland, it is conceivable that enhanced Cx expression downstream of Rho GTPase signaling drives normal morphogenesis during development while supporting metastasis during breast cancer progression. The effects of Cxs as upstream regulators of Rho GTPases, however, remain controversial, posing a challenge in defining the regulatory role of Cxs in Rho GTPase signaling within the mammary gland [198, 199, 201, 200]. We have recently delineated a role for Cx43 in regulating Rho GTPase signaling (unpublished data) and in establishing apical polarity and mitotic spindle orientation in 3-D cultures of human mammary epithelial cells (Bazzoun et al; submitted). Given the role of Rho GTPases in establishment and maintenance of epithelial apicobasal polarity and intercellular junctions and in regulation of cytoskeletal dynamics, and considering their developmental and tumorigenic roles in the mammary gland that overlap with those of Cxs, it becomes necessary to study the involvement of Rho GTPase signaling downstream of Cxs in the mammary gland.

7. Conclusion and Future Perspectives

Understanding the molecular events associated with the development and tumorigenesis of the mammary gland is key to establishing the appropriate preventive measures and treatment strategies for breast cancer. The loss of Cx expression and GJIC characterizes early stages of breast cancer. Studies investigating Cx expression profiles in patient tissues propose Cxs as independent prognostic markers, making Cxs potential therapeutic targets in breast cancer. Considering the channel-independent roles of Cxs and the diverse cellular events they regulate, elucidating the signaling pathways that link GJs to the development and tumorigenesis of the mammary gland would ensure a better targeted therapeutic approach in breast cancer. A cross-talk between Wnt pathways on one hand and GJs on the other hand is clearly illustrated in several tissues and biological contexts. Although independent regulatory roles are established for GJs and Wnt signaling in the development and tumorigenesis of the mammary gland, the link between the two pathways

in this tissue is poorly characterized. Our findings illustrate a role for Cxs in regulating Wnt signaling as a mechanism to drive development, maintain homeostasis and to suppress tumorigenesis of the mammary gland. We speculate the involvement of both canonical and noncanonical Wnt pathways as modulators of GJ functions in development of the mammary gland, and we implicate disruption of Wnt signaling as a result of altered Cx expression and function in breast cancer.

8. Figure Captions

Fig. 1

The canonical Wnt pathway. In the absence of a Wnt ligand **(a)**, the scaffolding proteins Axin and APC form a complex with the serine/threonine protein kinases CK1 and GSK-3 in the cytoplasm, referred to as the β-catenin destruction complex. CK1 and GSK-3 sequentially phosphorylate β-catenin, marking it for ubiquitination and subsequent proteasomal degradation, thereby reducing its nuclear translocation. Consequently, the TCF/LEF family of transcription factors acts as a transcriptional repressor by forming a complex with Gro/TLE, which interacts with HDACs to mediate chromatin compaction, causing the repression of the Wnt/β-catenin target genes. In the presence of Wnt **(b)**, the ligand binds to its receptor Fzd and coreceptor LRP5/6. The resulting complex recruits the scaffolding protein Dvl, which in turn recruits the β-catenin destruction complex. CK1 and GSK-3 phosphorylate LRP5/6, causing its activation and enhancing the recruitment of the β-catenin destruction complex. This results in the stabilization and accumulation of β-catenin in the cytoplasm, and its subsequent nuclear translocation. In the nucleus, β-catenin acts as a transcriptional coactivator by displacing Gro/TLE, thereby converting TCF/LEF into a transcriptional activator to induce the expression of the Wnt/β-catenin target genes and cell cycle progression

Fig. 2

A proposed model for the cross-talk between Cxs and Wnt/β-catenin signaling in the mammary gland. Depending on the context, Cxs may act as downstream "positive effectors" (red arrows) or as upstream "negative regulators" of the Wnt/β-catenin pathway (blue arrows) both in normal development and

tumorigenesis of the mammary gland (grey boxes). In normal development **(a)**, active canonical Wnt signaling induces Cx expression during morphogenesis and differentiation-driving events of the mammary gland. Cxs regulate the morphogenesis and differentiation of the tissue via channel-dependent and channel-independent mechanisms (red arrows) [54, 134, 136, 55, 137, 138, 52, 139]. Within a differentiated mammary gland, Cxs act as negative regulators of the Wnt/β-catenin pathway, a mechanism to sustain homeostasis and suppress tumorigenesis (blue arrows) [54, 56-58, 36]. In breast cancer **(b)**, the loss of Cx expression during early stages activates canonical Wnt signaling, which mediates hyperproliferation and primary tumor formation (blue arrows) [24]. Aberrant Wnt/β-catenin signaling induces Cx expression in advanced stages of breast cancer, supporting collective migration and tumor metastasis (red arrows) [53, 140]

Fig. 3

The noncanonical Wnt pathway. The PCP pathway **(a)** involves binding of a Wnt ligand to its receptor Fzd and coreceptor (ROR2, RYK, PTK7 or NRH1). The resulting complex recruits the scaffolding protein Dvl, which in turn recruits Daam1. This leads to GEF-mediated activation of Rho, which activates ROCK. Daam1 also mediates binding of profilin to actin. On the other hand, Dvl mediates Rac activation, which acts through activating JNK or independently. Profilin, ROCK and Rac regulate the dynamics of the actin and microtubule networks, which control cellular morphology, migration and division orientation. The Wnt/calcium pathway **(b)** involves the coactivation of Dvl and Fzd-associated G protein upon binding of a Wnt ligand, leading to the intracellular release of calcium. This results in PKC-mediated activation of Cdc42, which regulates actin dynamics. Calcium release also activates CaMKII, which activates the transcription factor NFAT. The Fzd-independent pathways **(c)** are triggered upon binding of a Wnt ligand to its receptor ROR2 or RYK. ROR2 subsequently mediates JNK activation, which regulates cell migration and convergent extension, among others. RYK controls axon guidance via the Src kinase family. In addition, the intracellular domain of RYK translocates to the nucleus upon cleavage by γ-secretase, where it mediates the expression of genes required for neuronal differentiation.

9. Conflict of Interest: The authors declare that they have no conflict of interest.

10. References

1. Chua AC, Hodson LJ, Moldenhauer LM, Robertson SA, Ingman WV. Dual roles for macrophages in ovarian cycle-associated development and remodelling of the mammary gland epithelium. Development. 2010;137(24):4229-38.
2. Gouon-Evans V, Rothenberg ME, Pollard JW. Postnatal mammary gland development requires macrophages and eosinophils. Development. 2000;127(11):2269-82.
3. Keely PJ, Wu JE, Santoro SA. The spatial and temporal expression of the α2β1 integrin and its ligands, collagen I, collagen IV, and laminin, suggest important roles in mouse mammary morphogenesis. Differentiation. 1995;59(1):1-13.
4. Koledova Z, Lu P. A 3D Fibroblast-Epithelium Co-culture Model for Understanding Microenvironmental Role in Branching Morphogenesis of the Mammary Gland. Mammary Gland Development. Springer; 2017. p. 217-31.
5. O'Brien J, Martinson H, Durand-Rougely C, Schedin P. Macrophages are crucial for epithelial cell death and adipocyte repopulation during mammary gland involution. Development. 2012;139(2):269-75.
6. Taddei I, Deugnier M-A, Faraldo MM, Petit V, Bouvard D, Medina D et al. β1 integrin deletion from the basal compartment of the mammary epithelium affects stem cells. Nature cell biology. 2008;10(6):716.
7. Woodward T, Mienaltowski A, Modi R, Bennett J, Haslam S. Fibronectin and the α5β1 integrin are under developmental and ovarian steroid regulation in the normal mouse mammary gland. Endocrinology. 2001;142(7):3214-22.
8. Insua-Rodríguez J, Oskarsson T. The extracellular matrix in breast cancer. Advanced drug delivery reviews. 2016;97:41-55.
9. Majidinia M, Yousefi B. Breast tumor stroma: A driving force in the development of resistance to therapies. Chemical biology & drug design. 2017;89(3):309-18.
10. Soysal SD, Tzankov A, Muenst SE. Role of the tumor microenvironment in breast cancer. Pathobiology. 2015;82(3-4):142-52.
11. Knudsen KA, Wheelock MJ. Cadherins and the mammary gland. Journal of cellular biochemistry. 2005;95(3):488-96.
12. McLachlan E, Shao Q, Laird DW. Connexins and gap junctions in mammary gland development and breast cancer progression. Journal of Membrane Biology. 2007;218(1-3):107-21.
13. Hirschi KK, Xu C, Tsukamoto T, Sager R. Gap junction genes Cx26 and Cx43 individually suppress the cancer phenotype of human mammary carcinoma cells and restore differentiation potential. Cell Growth and Differentiation-Publication American Association for Cancer Research. 1996;7(7):861-70.
14. Laird DW, Fistouris P, Batist G, Alpert L, Huynh HT, Carystinos GD et al. Deficiency of connexin43 gap junctions is an independent marker for breast tumors. Cancer research. 1999;59(16):4104-10.
15. Singal R, Tu Z, Vanwert J, Ginder G, Kiang D. Modulation of the connexin26 tumor suppressor gene expression through methylation in human mammary epithelial cell lines. Anticancer research. 2000;20(1A):59-64.
16. Qin H, Shao Q, Curtis H, Galipeau J, Belliveau DJ, Wang T et al. Retroviral delivery of connexin genes to human breast tumor cells inhibits in vivo tumor growth by a mechanism that is independent of significant gap junctional intercellular communication. Journal of Biological Chemistry. 2002;277(32):29132-8.
17. Kańczuga-Koda L, Sulkowska M, Koda M, Reszeć J, Famulski W, Baltaziak M et al. Expression of connexin 43 in breast cancer in comparison with mammary dysplasia and the normal mammary gland. Folia morphologica. 2003;62(4):439-42.
18. Momiyama M, Omori Y, Ishizaki Y, Nishikawa Y, Tokairin T, Ogawa J et al. Connexin26-mediated gap junctional communication reverses the malignant phenotype of MCF-7 breast cancer cells. Cancer science. 2003;94(6):501-7.
19. Shao Q, Wang H, McLachlan E, Veitch GI, Laird DW. Down-regulation of Cx43 by retroviral delivery of small interfering RNA promotes an aggressive breast cancer cell phenotype. Cancer research. 2005;65(7):2705-11.
20. Kalra J, Shao Q, Qin H, Thomas T, Alaoui-Jamali MA, Laird DW. Cx26 inhibits breast MDA-MB-435 cell tumorigenic properties by a gap junctional intercellular communication-independent mechanism. Carcinogenesis. 2006;27(12):2528-37.
21. Plante I, Laird DW. Decreased levels of connexin43 result in impaired development of the mammary gland in a mouse model of oculodentodigital dysplasia. Developmental biology. 2008;318(2):312-22.

22. Plante I, Wallis A, Shao Q, Laird DW. Milk secretion and ejection are impaired in the mammary gland of mice harboring a Cx43 mutant while expression and localization of tight and adherens junction proteins remain unchanged. Biology of reproduction. 2010;82(5):837-47.
23. Plante I, Stewart M, Barr K, Allan A, Laird D. Cx43 suppresses mammary tumor metastasis to the lung in a Cx43 mutant mouse model of human disease. Oncogene. 2011;30(14):1681.
24. Talhouk RS, Fares M-B, Rahme GJ, Hariri HH, Rayess T, Dbouk HA et al. Context dependent reversion of tumor phenotype by connexin-43 expression in MDA-MB231 cells and MCF-7 cells: Role of β-catenin/connexin43 association. Experimental cell research. 2013;319(20):3065-80.
25. Stewart MK, Plante I, Bechberger JF, Naus CC, Laird DW. Mammary gland specific knockdown of the physiological surge in Cx26 during lactation retains normal mammary gland development and function. PloS one. 2014;9(7):e101546.
26. Stewart MK, Bechberger JF, Welch I, Naus CC, Laird DW. Cx26 knockout predisposes the mammary gland to primary mammary tumors in a DMBA-induced mouse model of breast cancer. Oncotarget. 2015;6(35):37185.
27. Mroue R, Inman J, Mott J, Budunova I, Bissell MJ. Asymmetric expression of connexins between luminal epithelial-and myoepithelial-cells is essential for contractile function of the mammary gland. Developmental biology. 2015;399(1):15-26.
28. Ferrati S, Gadok AK, Brunaugh AD, Zhao C, Heersema LA, Smyth HD et al. Connexin membrane materials as potent inhibitors of breast cancer cell migration. Journal of The Royal Society Interface. 2017;14(133):20170313.
29. Monaghan P, Perusinghe N, Carlile G, Evans WH. Rapid modulation of gap junction expression in mouse mammary gland during pregnancy, lactation, and involution. Journal of Histochemistry & Cytochemistry. 1994;42(7):931-8.
30. Locke D, Perusinghe N, Newman T, Jayatilake H, Evans WH, Monaghan P. Developmental expression and assembly of connexins into homomeric and heteromeric gap junction hemichannels in the mouse mammary gland. Journal of cellular physiology. 2000;183(2):228-37.
31. Locke D, Stein T, Davies C, Morris J, Harris AL, Evans WH et al. Altered permeability and modulatory character of connexin channels during mammary gland development. Experimental cell research. 2004;298(2):643-60.
32. Locke D, Jamieson S, Stein T, Liu J, Hodgins MB, Harris AL et al. Nature of Cx30-containing channels in the adult mouse mammary gland. Cell and tissue research. 2007;328(1):97-107.
33. Talhouk RS, Elble RC, Bassam R, Daher M, Sfeir A, Mosleh LA et al. Developmental expression patterns and regulation of connexins in the mouse mammary gland: expression of connexin30 in lactogenesis. Cell and tissue research. 2005;319(1):49-59.
34. Dianati E, Poiraud J, Weber-Ouellette A, Plante I. Connexins, E-cadherin, Claudin-7 and β-catenin transiently form junctional nexuses during the post-natal mammary gland development. Developmental biology. 2016;416(1):52-68.
35. El-Sabban ME, Sfeir AJ, Daher MH, Kalaany NY, Bassam RA, Talhouk RS. ECM-induced gap junctional communication enhances mammary epithelial cell differentiation. Journal of Cell Science. 2003;116(17):3531-41.
36. Talhouk RS, Mroue R, Mokalled M, Abi-Mosleh L, Nehme R, Ismail A et al. Heterocellular interaction enhances recruitment of α and β-catenins and ZO-2 into functional gap-junction complexes and induces gap junction-dependant differentiation of mammary epithelial cells. Experimental cell research. 2008;314(18):3275-91.
37. van Genderen C, Okamura RM, Farinas I, Quo R-G, Parslow TG, Bruhn L et al. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. Genes & development. 1994;8(22):2691-703.
38. Fritz G, Just I, Kaina B. Rho GTPases are over-expressed in human tumors. International journal of cancer. 1999;81(5):682-7.
39. Fritz G, Brachetti C, Bahlmann F, Schmidt M, Kaina B. Rho GTPases in human breast tumours: expression and mutation analyses and correlation with clinical parameters. British journal of cancer. 2002;87(6):635.
40. Lin S-Y, Xia W, Wang JC, Kwong KY, Spohn B, Wen Y et al. β-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. Proceedings of the National Academy of Sciences. 2000;97(8):4262-6.
41. Imbert A, Eelkema R, Jordan S, Feiner H, Cowin P. ΔN89β-catenin induces precocious development, differentiation, and neoplasia in mammary gland. The Journal of cell biology. 2001;153(3):555-68.
42. Milovanovic T, Planutis K, Nguyen A, Marsh JL, Lin F, Hope C et al. Expression of Wnt genes and frizzled 1 and 2 receptors in normal breast epithelium and infiltrating breast carcinoma. International journal of oncology. 2004;25(5):1337-42.

43. Ewald AJ, Brenot A, Duong M, Chan BS, Werb Z. Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. Developmental cell. 2008;14(4):570-81.
44. Prasad CP, Mirza S, Sharma G, Prashad R, DattaGupta S, Rath G et al. Epigenetic alterations of CDH1 and APC genes: relationship with activation of Wnt/β-catenin pathway in invasive ductal carcinoma of breast. Life sciences. 2008;83(9-10):318-25.
45. Lindvall C, Zylstra CR, Evans N, West RA, Dykema K, Furge KA et al. The Wnt co-receptor Lrp6 is required for normal mouse mammary gland development. PloS one. 2009;4(6):e5813.
46. Raymond K, Cagnet S, Kreft M, Janssen H, Sonnenberg A, Glukhova MA. Control of mammary myoepithelial cell contractile function by α3β1 integrin signalling. The EMBO journal. 2011;30(10):1896-906.
47. Bray K, Gillette M, Young J, Loughran E, Hwang M, Sears JC et al. Cdc42 overexpression induces hyperbranching in the developing mammary gland by enhancing cell migration. Breast Cancer Research. 2013;15(5):R91.
48. Bagci H, Laurin M, Huber J, Muller W, Cote J. Impaired cell death and mammary gland involution in the absence of Dock1 and Rac1 signaling. Cell death & disease. 2014;5(8):e1375.
49. Van der Heyden M, Rook MB, Hermans M, Rijksen G, Boonstra J, Defize L et al. Identification of connexin43 as a functional target for Wnt signalling. Journal of Cell Science. 1998;111(12):1741-9.
50. Constantinou T, Baumann F, Lacher MD, Saurer S, Friis R, Dharmarajan A. SFRP-4 abrogates Wnt-3a-induced β-catenin and Akt/PKB signalling and reverses a Wnt-3a-imposed inhibition of in vitro mammary differentiation. Journal of Molecular Signaling. 2008;3(1):10.
51. Baxley SE, Jiang W, Serra R. Misexpression of wingless-related MMTV integration site 5A in mouse mammary gland inhibits the milk ejection response and regulates connexin43 phosphorylation. Biology of reproduction. 2011;85(5):907-15.
52. Wang H-X, Gillio-Meina C, Chen S, Gong X-Q, Li TY, Bai D et al. The canonical WNT2 pathway and FSH interact to regulate gap junction assembly in mouse granulosa cells. Biology of reproduction. 2013;89(2):39, 1-7.
53. Zhai Y, Wu R, Schwartz DR, Darrah D, Reed H, Kolligs FT et al. Role of β-catenin/T-cell factor-regulated genes in ovarian endometrioid adenocarcinomas. The American journal of pathology. 2002;160(4):1229-38.
54. Ai Z, Fischer A, Spray DC, Brown AM, Fishman GI. Wnt-1 regulation of connexin43 in cardiac myocytes. The Journal of clinical investigation. 2000;105(2):161-71.
55. Mureli S, Gans CP, Bare DJ, Geenen DL, Kumar NM, Banach K. Mesenchymal stem cells improve cardiac conduction by upregulation of connexin 43 through paracrine signaling. American Journal of Physiology-Heart and Circulatory Physiology. 2012;304(4):H600-H9.
56. Kamei J, Toyofuku T, Hori M. Negative regulation of p21 by β-catenin/TCF signaling: a novel mechanism by which cell adhesion molecules regulate cell proliferation. Biochemical and biophysical research communications. 2003;312(2):380-7.
57. Sirnes S, Bruun J, Kolberg M, Kjenseth A, Lind GE, Svindland A et al. Connexin43 acts as a colorectal cancer tumor suppressor and predicts disease outcome. International journal of cancer. 2012;131(3):570-81.
58. Yu SC, Xiao HL, Jiang XF, Wang QL, Li Y, Yang XJ et al. Connexin 43 Reverses Malignant Phenotypes of Glioma Stem Cells by Modulating E-Cadherin. Stem Cells. 2012;30(2):108-20.
59. Paine IS, Lewis MT. The terminal end bud: The little engine that could. Journal of mammary gland biology and neoplasia. 2017;22(2):93-108.
60. Musumeci G, Castrogiovanni P, Szychlinska MA, Aiello FC, Vecchio GM, Salvatorelli L et al. Mammary gland: From embryogenesis to adult life. Acta histochemica. 2015;117(4-5):379-85.
61. Veltmaat JM, Van Veelen W, Thiery JP, Bellusci S. Identification of the mammary line in mouse by Wnt10b expression. Developmental dynamics: an official publication of the American Association of Anatomists. 2004;229(2):349-56.
62. Gjorevski N, Nelson CM. Integrated morphodynamic signalling of the mammary gland. Nature reviews Molecular cell biology. 2011;12(9):581.
63. Brisken C, O'Malley B. Hormone action in the mammary gland. Cold Spring Harbor perspectives in biology. 2010:a003178.
64. Parsa S, Ramasamy SK, De Langhe S, Gupte VV, Haigh JJ, Medina D et al. Terminal end bud maintenance in mammary gland is dependent upon FGFR2b signaling. Developmental biology. 2008;317(1):121-31.
65. Hinck L, Silberstein GB. Key stages in mammary gland development: the mammary end bud as a motile organ. Breast Cancer Research. 2005;7(6):245.
66. Atwood C, Hovey R, Glover J, Chepko G, Ginsburg E, Robison W et al. Progesterone induces side-branching of the ductal epithelium in the mammary glands of peripubertal mice. Journal of Endocrinology. 2000;167(1):39-52.

67. Brisken C. Hormonal control of alveolar development and its implications for breast carcinogenesis. Journal of mammary gland biology and neoplasia. 2002;7(1):39-48.
68. Oakes SR, Rogers RL, Naylor MJ, Ormandy CJ. Prolactin regulation of mammary gland development. Journal of mammary gland biology and neoplasia. 2008;13(1):13-28.
69. Howard BA, Gusterson BA. Human breast development. Journal of mammary gland biology and neoplasia. 2000;5(2):119-37.
70. Zhu J, Xiong G, Trinkle C, Xu R. Integrated extracellular matrix signaling in mammary gland development and breast cancer progression. Histology and histopathology. 2014;29(9):1083.
71. Xu R, Boudreau A, Bissell MJ. Tissue architecture and function: dynamic reciprocity via extra-and intra-cellular matrices. Cancer and metastasis reviews. 2009;28(1-2):167-76.
72. Talhouk RS, Bissell MJ, Werb Z. Coordinated expression of extracellular matrix-degrading proteinases and their inhibitors regulates mammary epithelial function during involution. The Journal of Cell Biology. 1992;118(5):1271-82.
73. Wiseman BS, Sternlicht MD, Lund LR, Alexander CM, Mott J, Bissell MJ et al. Site-specific inductive and inhibitory activities of MMP-2 and MMP-3 orchestrate mammary gland branching morphogenesis. The Journal of cell biology. 2003;162(6):1123-33.
74. Dermietzel R, Hwang T, Spray D. The gap junction family: structure, function and chemistry. Anatomy and embryology. 1990;182(6):517-28.
75. Söhl G, Willecke K. Gap junctions and the connexin protein family. Cardiovascular research. 2004;62(2):228-32.
76. Dbouk HA, Mroue RM, El-Sabban ME, Talhouk RS. Connexins: a myriad of functions extending beyond assembly of gap junction channels. Cell Communication and signaling. 2009;7(1):4.
77. Leithe E, Mesnil M, Aasen T. The connexin 43 C-terminus: A tail of many tales. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2017.
78. Su V, Lau AF. Connexins: mechanisms regulating protein levels and intercellular communication. FEBS letters. 2014;588(8):1212-20.
79. Kelly JJ, Simek J, Laird DW. Mechanisms linking connexin mutations to human diseases. Cell and tissue research. 2015;360(3):701-21.
80. Naus CC, Laird DW. Implications and challenges of connexin connections to cancer. Nature Reviews Cancer. 2010;10(6):435.
81. El-Saghir JA, El-Habre ET, El-Sabban ME, Talhouk RS. Connexins: a junctional crossroad to breast cancer. International Journal of Developmental Biology. 2011;55(7-8-9):773-80.
82. Monaghan P, Clarke C, Perusinghe NP, Moss DW, Chen X-Y, Evans WH. Gap junction distribution and connexin expression in human breast. Experimental cell research. 1996;223(1):29-38.
83. Jamieson S, Going JJ, D'Arcy R, George WD. Expression of gap junction proteins connexin 26 and connexin 43 in normal human breast and in breast tumours. The Journal of pathology. 1998;184(1):37-43.
84. Pozzi A, Risek B, Kiang DT, Gilula NB, Kumar NM. Analysis of multiple gap junction gene products in the rodent and human mammary gland. Experimental cell research. 1995;220(1):212-9.
85. Lambe T, Finlay D, Murphy M, Martin F. Differential expression of connexin 43 in mouse mammary cells. Cell biology international. 2006;30(5):472-9.
86. Reaume AG, de Sousa PA, Kulkarni S, Langille BL, Zhu D, Davies TC et al. Cardiac malformation in neonatal mice lacking connexin43. Science. 1995;267(5205):1831-4.
87. Gabriel H-D, Jung D, Bützler C, Temme A, Traub O, Winterhager E et al. Transplacental uptake of glucose is decreased in embryonic lethal connexin26-deficient mice. The Journal of cell biology. 1998;140(6):1453-61.
88. Stewart MK, Gong X-Q, Barr KJ, Bai D, Fishman GI, Laird DW. The severity of mammary gland developmental defects is linked to the overall functional status of Cx43 as revealed by genetically modified mice. Biochemical Journal. 2013;449(2):401-13.
89. Plum A, Hallas G, Magin T, Dombrowski F, Hagendorff A, Schumacher B et al. Unique and shared functions of different connexins in mice. Current biology. 2000;10(18):1083-91.
90. Winterhager E, Pielensticker N, Freyer J, Ghanem A, Schrickel JW, Kim J-S et al. Replacement of connexin43 by connexin26 in transgenic mice leads to dysfunctional reproductive organs and slowed ventricular conduction in the heart. BMC developmental biology. 2007;7(1):26.
91. Bry C, Maass K, Miyoshi K, Willecke K, Ott T, Robinson GW et al. Loss of connexin 26 in mammary epithelium during early but not during late pregnancy results in unscheduled apoptosis and impaired development. Developmental biology. 2004;267(2):418-29.

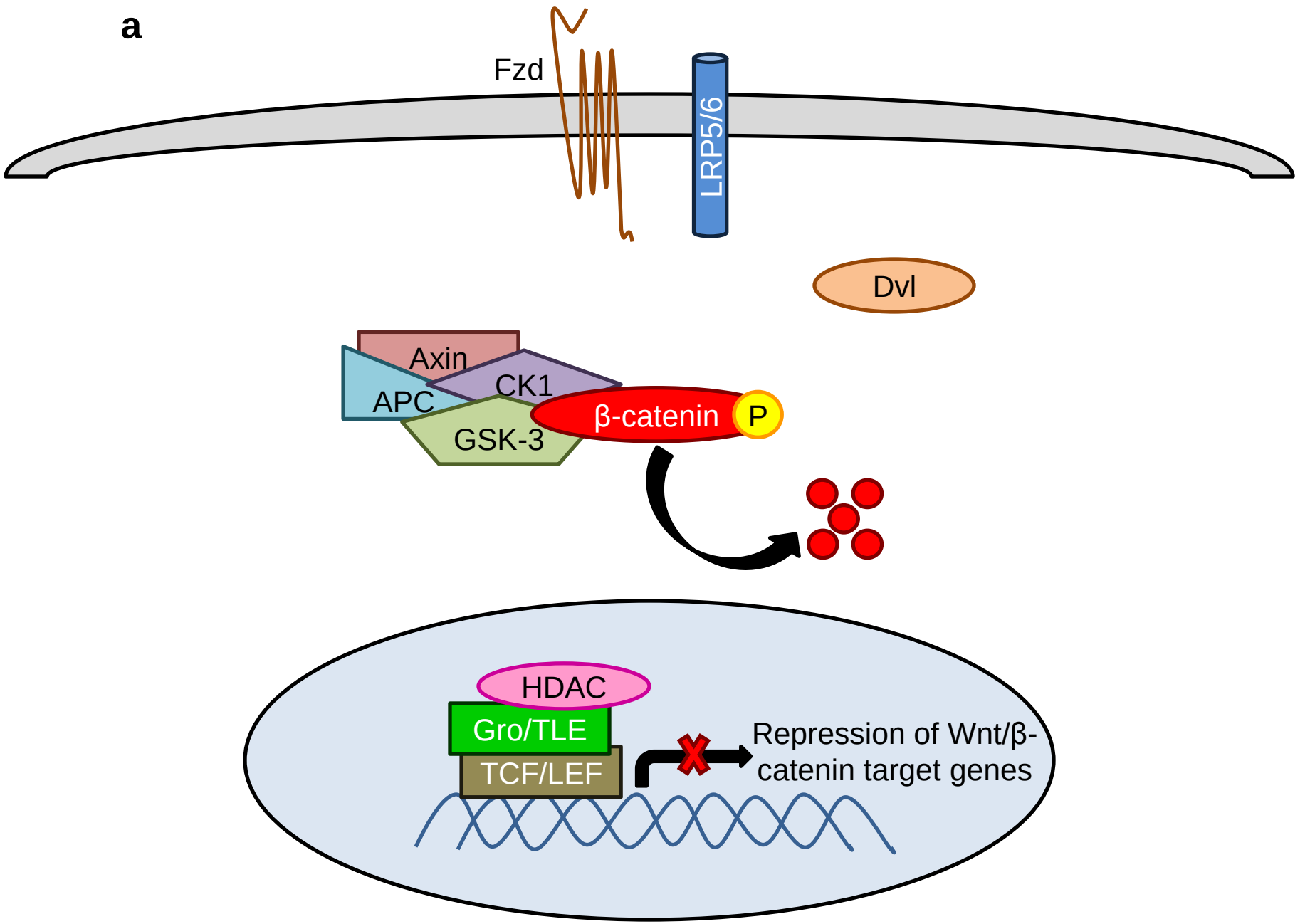
92. Ormandy CJ, Naylor M, Harris J, Robertson F, Horseman ND, Lindeman GJ et al. Investigation of the transcriptional changes underlying functional defects in the mammary glands of prolactin receptor knockout mice. Recent Progress in Hormone Research. 2003;58:297-324.
93. Mroue R, El-Sabban M, Talhouk R. Connexins and the gap in context. Integrative Biology. 2011;3(4):255-66.
94. Talhouk RS, Khalil AA, Bajjani R, Rahme GJ, El-Sabban ME. Gap junctions mediate STAT5-independent β-casein expression in CID-9 mammary epithelial cells. Cell communication & adhesion. 2011;18(5):104-16.
95. Teleki I, Szasz AM, Maros ME, Gyorffy B, Kulka J, Meggyeshazi N et al. Correlations of differentially expressed gap junction connexins Cx26, Cx30, Cx32, Cx43 and Cx46 with breast cancer progression and prognosis. PloS one. 2014;9(11):e112541.
96. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. Developmental cell. 2009;17(1):9-26.
97. Zardawi SJ, O'Toole SA, Sutherland RL, Musgrove EA. Dysregulation of Hedgehog, Wnt and Notch signalling pathways in breast cancer. Histology and histopathology. 2009;24(3):385-98.
98. Rao TP, Kühl M. An updated overview on Wnt signaling pathways: a prelude for more. Circulation research. 2010;106(12):1798-806.
99. Turashvili G, Bouchal J, Burkadze G, Kolar Z. Wnt signaling pathway in mammary gland development and carcinogenesis. Pathobiology. 2006;73(5):213-23.
100. Incassati A, Chandramouli A, Eelkema R, Cowin P. Key signaling nodes in mammary gland development and cancer: β-catenin. Breast Cancer Research. 2010;12(6):213.
101. Yu QC, Verheyen EM, Zeng YA. Mammary development and breast cancer: a Wnt perspective. Cancers. 2016;8(7):65.
102. Boras-Granic K, Hamel PA. Wnt-signalling in the embryonic mammary gland. Journal of mammary gland biology and neoplasia. 2013;18(2):155-63.
103. Jarde T, Dale T. Wnt signalling in murine postnatal mammary gland development. Acta physiologica. 2012;204(1):118-27.
104. Cai C, Yu QC, Jiang W, Liu W, Song W, Yu H et al. R-spondin1 is a novel hormone mediator for mammary stem cell self-renewal. Genes & development. 2014:gad. 245142.114.
105. Chu EY, Hens J, Andl T, Kairo A, Yamaguchi TP, Brisken C et al. Canonical WNT signaling promotes mammary placode development and is essential for initiation of mammary gland morphogenesis. Development. 2004;131(19):4819-29.
106. Kouros-Mehr H, Werb Z. Candidate regulators of mammary branching morphogenesis identified by genome-wide transcript analysis. Developmental dynamics: an official publication of the American Association of Anatomists. 2006;235(12):3404-12.
107. Lane TF, Leder P. Wnt-10b directs hypermorphic development and transformation in mammary glands of male and female mice. Oncogene. 1997;15(18):2133.
108. Weber-Hall SJ, Phippard DJ, Niemeyer CC, Dale TC. Developmental and hormonal regulation of Wnt gene expression in the mouse mammary gland. Differentiation. 1994;57(3):205-14.
109. Roarty K, Shore AN, Creighton CJ, Rosen JM. Ror2 regulates branching, differentiation, and actin-cytoskeletal dynamics within the mammary epithelium. J Cell Biol. 2015;208(3):351-66.
110. Ji H, Goode RJ, Vaillant F, Mathivanan S, Kapp EA, Mathias RA et al. Proteomic profiling of secretome and adherent plasma membranes from distinct mammary epithelial cell subpopulations. Proteomics. 2011;11(20):4029-39.
111. Lindvall C, Evans NC, Zylstra CR, Li Y, Alexander CM, Williams BO. The Wnt signaling receptor Lrp5 is required for mammary ductal stem cell activity and Wnt1-induced tumorigenesis. Journal of Biological Chemistry. 2006;281(46):35081-7.
112. Brisken C, Heineman A, Chavarria T, Elenbaas B, Tan J, Dey SK et al. Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling. Genes & development. 2000;14(6):650-4.
113. Dolled-Filhart M, McCabe A, Giltnane J, Cregger M, Camp RL, Rimm DL. Quantitative in situ analysis of β-catenin expression in breast cancer shows decreased expression is associated with poor outcome. Cancer research. 2006;66(10):5487-94.
114. Sørlie T, Bukholm I, Børresen-Dale A. Truncating somatic mutation in exon 15 of the APC gene is a rare event in human breast carcinomas. Mutations in brief no. 179. Online. Human mutation. 1998;12(3):215-.
115. Schlosshauer PW, Brown SA, Eisinger K, Yan Q, Guglielminetti ER, Parsons R et al. APC truncation and increased β-catenin levels in a human breast cancer cell line. Carcinogenesis. 2000;21(7):1453-6.

116. Jönsson M, Borg Å, Nilbert M, Andersson T. Involvement of adenomatous polyposis coli (APC)/β-catenin signalling in human breast cancer. European journal of cancer. 2000;36(2):242-8.
117. Sawyer EJ, Hanby AM, Rowan AJ, Gillett CE, Thomas RE, Poulsom R et al. The Wnt pathway, epithelial–stromal interactions, and malignant progression in phyllodes tumours. The Journal of pathology. 2002;196(4):437-44.
118. Abraham SC, Reynolds C, Lee J-H, Montgomery EA, Baisden BL, Krasinskas AM et al. Fibromatosis of the breast and mutations involving the APC/β-catenin pathway. Human pathology. 2002;33(1):39-46.
119. Ozaki S, Ikeda S, Ishizaki Y, Kurihara T, Tokumoto N, Iseki M et al. Alterations and correlations of the components in the Wnt signaling pathway and its target genes in breast cancer. Oncology reports. 2005;14(6):1437-43.
120. Hayes MJ, Thomas D, Emmons A, Giordano TJ, Kleer CG. Genetic changes of Wnt pathway genes are common events in metaplastic carcinomas of the breast. Clinical Cancer Research. 2008;14(13):4038-44.
121. Huguet EL, McMahon JA, McMahon AP, Bicknell R, Harris AL. Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue. Cancer research. 1994;54(10):2615-21.
122. Lejeune S, Huguet EL, Hamby A, Poulsom R, Harris AL. Wnt5a cloning, expression, and up-regulation in human primary breast cancers. Clinical Cancer Research. 1995;1(2):215-22.
123. Björklund P, Svedlund J, Olsson A-K, Åkerström G, Westin G. The internally truncated LRP5 receptor presents a therapeutic target in breast cancer. PloS one. 2009;4(1):e4243.
124. Liu C-C, Prior J, Piwnica-Worms D, Bu G. LRP6 overexpression defines a class of breast cancer subtype and is a target for therapy. Proceedings of the National Academy of Sciences. 2010;107(11):5136-41.
125. Nagahata T, Shimada T, Harada A, Nagai H, Onda M, Yokoyama S et al. Amplification, up-regulation and over-expression of DVL-1, the human counterpart of the Drosophila disheveled gene, in primary breast cancers. Cancer science. 2003;94(6):515-8.
126. Van der Auwera I, Van Laere SJ, Van den Bosch S, Van den Eynden G, Trinh B, Van Dam P et al. Aberrant methylation of the Adenomatous Polyposis Coli (APC) gene promoter is associated with the inflammatory breast cancer phenotype. British journal of cancer. 2008;99(10):1735.
127. Ai L, Tao Q, Zhong S, Fields CR, Kim W-J, Lee MW et al. Inactivation of Wnt inhibitory factor-1 (WIF1) expression by epigenetic silencing is a common event in breast cancer. Carcinogenesis. 2006;27(7):1341-8.
128. Suzuki H, Toyota M, Caraway H, Gabrielson E, Ohmura T, Fujikane T et al. Frequent epigenetic inactivation of Wnt antagonist genes in breast cancer. British journal of cancer. 2008;98(6):1147.
129. Shulewitz M, Soloviev I, Wu T, Koeppen H, Polakis P, Sakanaka C. Repressor roles for TCF-4 and Sfrp1 in Wnt signaling in breast cancer. Oncogene. 2006;25(31):4361.
130. Bivi N, Pacheco-Costa R, Brun LR, Murphy TR, Farlow NR, Robling AG et al. Absence of Cx43 selectively from osteocytes enhances responsiveness to mechanical force in mice. Journal of Orthopaedic Research. 2013;31(7):1075-81.
131. Rinaldi F, Hartfield E, Crompton L, Badger J, Glover C, Kelly C et al. Cross-regulation of Connexin43 and β-catenin influences differentiation of human neural progenitor cells. Cell death & disease. 2015;5(1):e1017.
132. Pacheco-Costa R, Kadakia JR, Atkinson EG, Wallace JM, Plotkin LI, Reginato RD. Connexin37 deficiency alters organic bone matrix, cortical bone geometry, and increases Wnt/β-catenin signaling. Bone. 2017;97:105-13.
133. Ishikawa M, Iwamoto T, Fukumoto S, Yamada Y. Pannexin 3 inhibits proliferation of osteoprogenitor cells by regulating Wnt and p21 signaling. Journal of Biological Chemistry. 2014;289(5):2839-51.
134. Czyz J, Guan K, Zeng Q, Wobus AM. Loss of beta1 integrin function results in upregulation of connexin expression in embryonic stem cell-derived cardiomyocytes. International Journal of Developmental Biology. 2003;49(1):33-41.
135. Du W, Li J, Du W, Li J, Wang Q, Hou J et al. Lithium Chloride Regulates Connexin43 in Skeletal Myoblasts In Vitro: Possible Involvement in Wnt/β-Catenin Signaling. Cell communication & adhesion. 2008;15(3):261-71.
136. Heo JS, Lee JC. β-catenin mediates cyclic strain-stimulated cardiomyogenesis in mouse embryonic stem cells through ROS-dependent and integrin-mediated PI3K/Akt pathways. Journal of cellular biochemistry. 2011;112(7):1880-9.
137. Guger KA, Gumbiner BM. β-Catenin Has Wnt-like Activity and Mimics the Nieuwkoop Signaling Center inXenopusDorsal–Ventral Patterning. Developmental biology. 1995;172(1):115-25.
138. Samarzija I, Sini P, Schlange T, MacDonald G, Hynes NE. Wnt3a regulates proliferation and migration of HUVEC via canonical and non-canonical Wnt signaling pathways. Biochemical and biophysical research communications. 2009;386(3):449-54.

139. Prunskaite-Hyyryläinen R, Shan J, Railo A, Heinonen KM, Miinalainen I, Yan W et al. Wnt4, a pleiotropic signal for controlling cell polarity, basement membrane integrity, and antimüllerian hormone expression during oocyte maturation in the female follicle. The FASEB Journal. 2014;28(4):1568-81.
140. Umazume K, Tsukahara R, Liu L, de Castro JPF, McDonald K, Kaplan HJ et al. Role of retinal pigment epithelial cell β-catenin signaling in experimental proliferative vitreoretinopathy. The American Journal of Pathology. 2014;184(5):1419-28.
141. Phillips SL, Williams CB, Zambrano JN, Williams CJ, Yeh ES. Connexin 43 in the development and progression of breast cancer: What's the connection? International journal of oncology. 2017;51(4):1005-13.
142. Banerjee D. Connexin's connection in breast cancer growth and progression. International journal of cell biology. 2016;2016.
143. Komiya Y, Habas R. Wnt signal transduction pathways. Organogenesis. 2008;4(2):68-75.
144. Segalen M, Bellaïche Y, editors. Cell division orientation and planar cell polarity pathways. Seminars in cell & developmental biology; 2009: Elsevier.
145. Gómez-Orte E, Sáenz-Narciso B, Moreno S, Cabello J. Multiple functions of the noncanonical Wnt pathway. Trends in genetics. 2013;29(9):545-53.
146. Sokol SY, editor. Spatial and temporal aspects of Wnt signaling and planar cell polarity during vertebrate embryonic development. Seminars in cell & developmental biology; 2015: Elsevier.
147. Sedgwick AE, D'Souza-Schorey C. Wnt signaling in cell motility and invasion: drawing parallels between development and cancer. Cancers. 2016;8(9):80.
148. Dunn NR, Tolwinski NS. Ptk7 and Mcc, unfancied components in non-canonical wnt signaling and cancer. Cancers. 2016;8(7):68.
149. De A. Wnt/Ca2+ signaling pathway: a brief overview. Acta biochimica et biophysica Sinica. 2011;43(10):745-56.
150. Green J, Nusse R, van Amerongen R. The role of Ryk and Ror receptor tyrosine kinases in Wnt signal transduction. Cold Spring Harbor perspectives in biology. 2013:a009175.
151. Angers S, Moon RT. Proximal events in Wnt signal transduction. Nature reviews Molecular cell biology. 2009;10(7):468.
152. Debebe Z, Rathmell WK. Ror2 as a therapeutic target in cancer. Pharmacology & therapeutics. 2015;150:143-8.
153. Clark CE, Nourse CC, Cooper HM. The tangled web of non-canonical Wnt signalling in neural migration. Neurosignals. 2012;20(3):202-20.
154. Etienne-Manneville S, Hall A. Rho GTPases in cell biology. Nature. 2002;420(6916):629.
155. Schlessinger K, Hall A, Tolwinski N. Wnt signaling pathways meet Rho GTPases. Genes & development. 2009;23(3):265-77.
156. Hanna S, El-Sibai M. Signaling networks of Rho GTPases in cell motility. Cellular signalling. 2013;25(10):1955-61.
157. Mack NA, Georgiou M. The interdependence of the Rho GTPases and apicobasal cell polarity. Small GTPases. 2014;5(2):e973768.
158. Burbelo P, Wellstein A, Pestell RG. Altered Rho GTPase signaling pathways in breast cancer cells. Breast cancer research and treatment. 2004;84(1):43-8.
159. Zuo Y, Oh W, Ulu A, Frost JA. Minireview: mouse models of Rho GTPase function in mammary gland development, tumorigenesis, and metastasis. Molecular Endocrinology. 2016;30(3):278-89.
160. Bray K, Brakebusch C, Vargo-Gogola T. The Rho GTPase Cdc42 is required for primary mammary epithelial cell morphogenesis in vitro. Small GTPases. 2011;2(5):247-58.
161. Druso JE, Endo M, Lin M-cJ, Peng X, Antonyak MA, Meller S et al. An essential role for Cdc42 in the functioning of the adult mammary gland. Journal of Biological Chemistry. 2016;291(17):8886-95.
162. Ahn S-J, Chung K-W, Lee R-A, Park I-A, Lee S-H, Park DE et al. Overexpression of βPix-a in human breast cancer tissues. Cancer letters. 2003;193(1):99-107.
163. Lane J, Martin TA, Mansel RE, Jiang WG, editors. The expression and prognostic value of the guanine nucleotide exchange factors (GEFs) Trio, Vav1 and TIAM-1 in human breast cancer. International Seminars in Surgical Oncology; 2008: BioMed Central.
164. Hanna S, Khalil B, Nasrallah A, Saykali BA, Sobh R, Nasser S et al. StarD13 is a tumor suppressor in breast cancer that regulates cell motility and invasion. International journal of oncology. 2014;44(5):1499-511.
165. El-Sibai M, Pertz O, Pang H, Yip S-C, Lorenz M, Symons M et al. RhoA/ROCK-mediated switching between Cdc42- and Rac1-dependent protrusion in MTLn3 carcinoma cells. Experimental cell research. 2008;314(7):1540-52.
166. El-Sibai M, Nalbant P, Pang H, Flinn RJ, Sarmiento C, Macaluso F et al. Cdc42 is required for EGF-stimulated protrusion and motility in MTLn3 carcinoma cells. Journal of cell science. 2007;120(19):3465-74.

167. Pillé J-Y, Denoyelle C, Varet J, Bertrand J-R, Soria J, Opolon P et al. Anti-RhoA and anti-RhoC siRNAs inhibit the proliferation and invasiveness of MDA-MB-231 breast cancer cells in vitro and in vivo. Molecular therapy. 2005;11(2):267-74.
168. Wyckoff JB, Pinner SE, Gschmeissner S, Condeelis JS, Sahai E. ROCK-and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion in vivo. Current Biology. 2006;16(15):1515-23.
169. Bravo-Cordero JJ, Sharma VP, Roh-Johnson M, Chen X, Eddy R, Condeelis J et al. Spatial regulation of RhoC activity defines protrusion formation in migrating cells. J Cell Sci. 2013:jcs. 123547.
170. Bravo-Cordero JJ, Oser M, Chen X, Eddy R, Hodgson L, Condeelis J. A novel spatiotemporal RhoC activation pathway locally regulates cofilin activity at invadopodia. Current Biology. 2011;21(8):635-44.
171. Moshfegh Y, Bravo-Cordero JJ, Miskolci V, Condeelis J, Hodgson L. A Trio–Rac1–Pak1 signalling axis drives invadopodia disassembly. Nature cell biology. 2014;16(6):571.
172. Yip S-C, El-Sibai M, Coniglio SJ, Mouneimne G, Eddy RJ, Drees BE et al. The distinct roles of Ras and Rac in PI 3-kinase-dependent protrusion during EGF-stimulated cell migration. Journal of cell science. 2007;120(17):3138-46.
173. Zhao X, Lu L, Pokhriyal N, Ma H, Duan L, Lin S et al. Overexpression of RhoA induces preneoplastic transformation of primary mammary epithelial cells. Cancer research. 2009;69(2):483-91.
174. Chan C-H, Lee S-W, Li C-F, Wang J, Yang W-L, Wu C-Y et al. Deciphering the transcriptional complex critical for RhoA gene expression and cancer metastasis. Nature cell biology. 2010;12(5):457.
175. Castillo-Pichardo L, Humphries-Bickley T, De La Parra C, Forestier-Roman I, Martinez-Ferrer M, Hernandez E et al. The Rac inhibitor EHop-016 inhibits mammary tumor growth and metastasis in a nude mouse model. Translational oncology. 2014;7(5):546-55.
176. Citi S, Guerrera D, Spadaro D, Shah J. Epithelial junctions and Rho family GTPases: the zonular signalosome. Small GTPases. 2014;5(4):e973760.
177. Yagi S, Matsuda M, Kiyokawa E. Suppression of Rac1 activity at the apical membrane of MDCK cells is essential for cyst structure maintenance. EMBO reports. 2012;13(3):237-43.
178. Timmerman I, Heemskerk N, Kroon J, Schaefer A, van Rijssel J, Hoogenboezem M et al. A local VE-cadherin and Trio-based signaling complex stabilizes endothelial junctions through Rac1. J Cell Sci. 2015;128(16):3041-54.
179. Katayama K-i, Melendez J, Baumann JM, Leslie JR, Chauhan BK, Nemkul N et al. Loss of RhoA in neural progenitor cells causes the disruption of adherens junctions and hyperproliferation. Proceedings of the National Academy of Sciences. 2011;108(18):7607-12.
180. Herder C, Swiercz JM, Müller C, Peravali R, Quiring R, Offermanns S et al. ArhGEF18 regulates RhoA-Rock2 signaling to maintain neuro-epithelial apico-basal polarity and proliferation. Development. 2013;140(13):2787-97.
181. Wu X, Li S, Chrostek-Grashoff A, Czuchra A, Meyer H, Yurchenco PD et al. Cdc42 is crucial for the establishment of epithelial polarity during early mammalian development. Developmental Dynamics. 2007;236(10):2767-78.
182. Wei L, Taffet GE, Khoury DS, Bo J, Li Y, Yatani A et al. Disruption of Rho signaling results in progressive atrioventricular conduction defects while ventricular function remains preserved. The FASEB journal. 2004;18(7):857-9.
183. Maddala R, Deng P-F, Costello JM, Wawrousek EF, Zigler JS, Rao VP. Impaired cytoskeletal organization and membrane integrity in lens fibers of a Rho GTPase functional knockout transgenic mouse. Laboratory investigation. 2004;84(6):679.
184. Wang L, Liu S, Zhang H, Hu S, Wei Y. RhoA activity increased in myocardium of arrhythmogenic cardiomyopathy patients and affected connexin 43 protein expression in HL-1 cells. International journal of clinical and experimental medicine. 2015;8(8):12906.
185. Matsuda T, Fujio Y, Nariai T, Ito T, Yamane M, Takatani T et al. N-cadherin signals through Rac1 determine the localization of connexin 43 in cardiac myocytes. Journal of molecular and cellular cardiology. 2006;40(4):495-502.
186. Van Hengel J, D'Hooge P, Hooghe B, Wu X, Libbrecht L, De Vos R et al. Continuous cell injury promotes hepatic tumorigenesis in cdc42-deficient mouse liver. Gastroenterology. 2008;134(3):781-92.
187. Anderson SC, Stone C, Tkach L, SundarRaj N. Rho and Rho-kinase (ROCK) signaling in adherens and gap junction assembly in corneal epithelium. Investigative ophthalmology & visual science. 2002;43(4):978-86.
188. Derangeon M, Bourmeyster N, Plaisance I, Pinet-Charvet C, Chen Q, Duthe F et al. RhoA GTPase and F-actin dynamically regulate the permeability of Cx43-made channels in rat cardiac myocytes. Journal of Biological Chemistry. 2008;283(45):30754-65.
189. Geletu M, Guy S, Greer S, Raptis L. Differential effects of polyoma virus middle tumor antigen mutants upon gap junctional, intercellular communication. Experimental cell research. 2015;336(2):223-31.

190. Ito S, Ito Y, Senga T, Hattori S, Matsuo S, Hamaguchi M. v-Src requires Ras signaling for the suppression of gap junctional intercellular communication. Oncogene. 2006;25(16):2420.
191. Stains JP, Civitelli R. Gap junctions regulate extracellular signal-regulated kinase signaling to affect gene transcription. Molecular biology of the cell. 2005;16(1):64-72.
192. Somekawa S, Fukuhara S, Nakaoka Y, Fujita H, Saito Y, Mochizuki N. Enhanced functional gap junction neoformation by protein kinase A–dependent and Epac-dependent signals downstream of cAMP in cardiac myocytes. Circulation research. 2005;97(7):655-62.
193. Hayashi T, Nomata K, Chang C-C, Ruch RJ, Trosko JE. Cooperative effects of v-myc and c-Ha-ras oncogenes on gap junctional intercellular communication and tumorigenicity in rat liver epithelial cells. Cancer letters. 1998;128(2):145-54.
194. Chen X, Shuzo O, Li Y, Han R. Effect of d-limonene, Salvia miltiorrhiza and turmeric derivatives on membrane association of Ras gene product and gap junction intercellular communication. Yao xue xue bao= Acta pharmaceutica Sinica. 1998;33(11):821-7.
195. Brownell HL, Whitfield JF, Raptis L. Cellular Ras partly mediates gap junction closure by the polyoma virus middle Tumor antigen. Cancer letters. 1996;103(1):99-106.
196. Brownell HL, Whitfield JF, Raptis L. Elimination of intercellular junctional communication requires lower Ras (leu61) levels than stimulation of anchorage-independent proliferation. Cancer detection and prevention. 1997;21(4):289-94.
197. BROWNELL HL, NARSIMHAN RP, CORBLEY MJ, MANN VM, WHITFIELD JF, RAPTIS L. Ras is involved in gap junction closure in proliferating fibroblasts or preadipocytes but not in differentiated adipocytes. DNA and cell biology. 1996;15(6):443-51.
198. Zhang J, Yang G-m, Zhu Y, Peng X-y, Liu L-m, Li T. Bradykinin induces vascular contraction after hemorrhagic shock in rats. journal of surgical research. 2015;193(1):334-43.
199. Sin W-C, Tse M, Planque N, Perbal B, Lampe PD, Naus CC. Matricellular protein CCN3 (NOV) regulates actin cytoskeleton reorganization. Journal of Biological Chemistry. 2009;284(43):29935-44.
200. Liu X, Hashimoto-Torii K, Torii M, Ding C, Rakic P. Gap junctions/hemichannels modulate interkinetic nuclear migration in the forebrain precursors. Journal of Neuroscience. 2010;30(12):4197-209.
201. Mendoza-Naranjo A, Cormie P, Serrano AE, Hu R, O'Neill S, Wang CM et al. Targeting Cx43 and N-cadherin, which are abnormally upregulated in venous leg ulcers, influences migration, adhesion and activation of Rho GTPases. PloS one. 2012;7(5):e37374.
202. Machtaler S, Choi K, Dang-Lawson M, Falk L, Pournia F, Naus CC et al. The role of the gap junction protein connexin43 in B lymphocyte motility and migration. FEBS letters. 2014;588(8):1249-58.
203. Machtaler S, Dang-Lawson M, Choi K, Jang C, Naus CC, Matsuuchi L. The gap junction protein Cx43 regulates B-lymphocyte spreading and adhesion. J Cell Sci. 2011;124(15):2611-21.


a
Fzd
LRP5/6
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Axin
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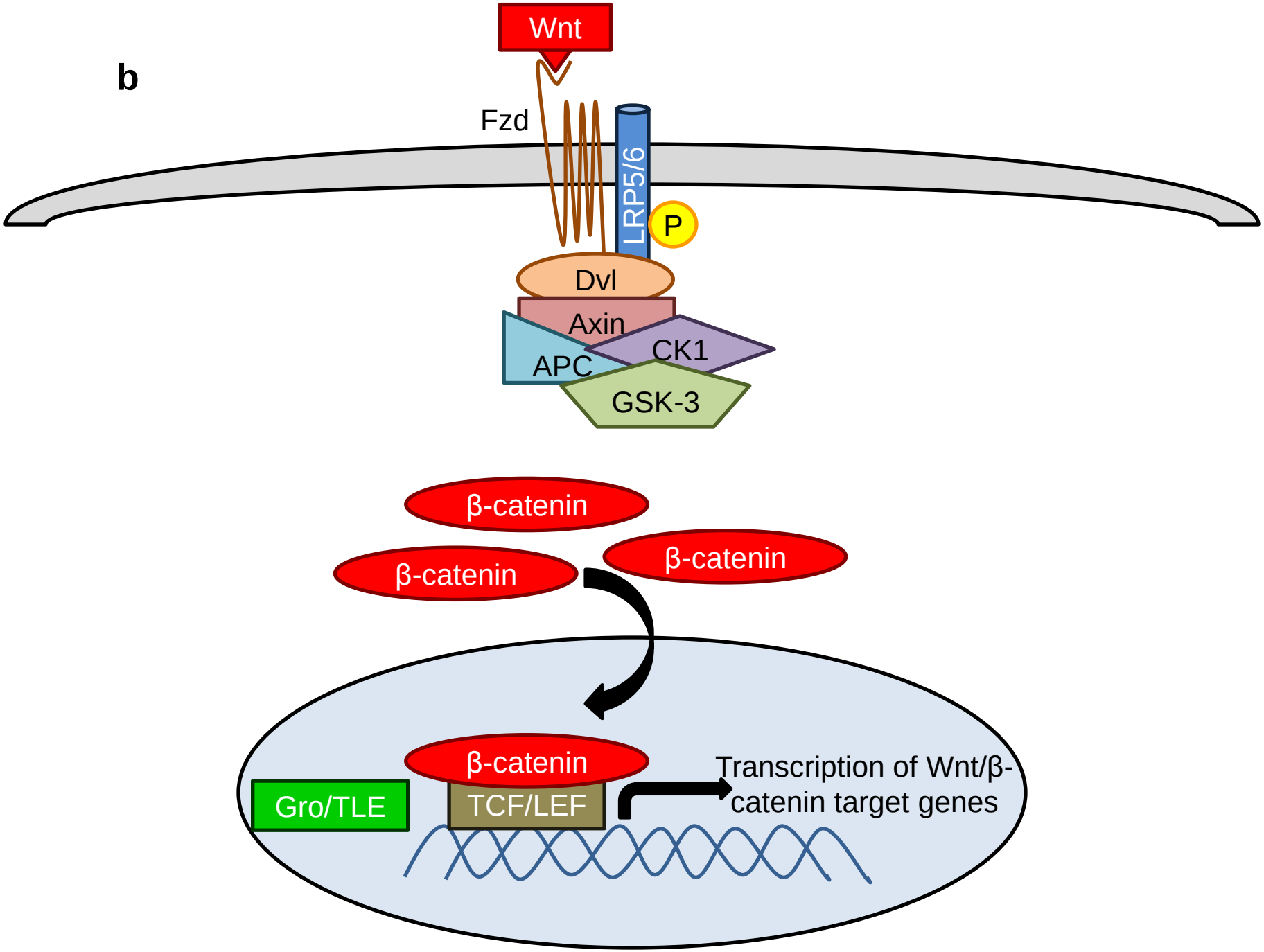
HDAC
Gro/TLE
TCF/LEF
Repression of Wnt/β-catenin target genes


b
Wnt
Fzd
LRP5/6
P
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Axin
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CK1
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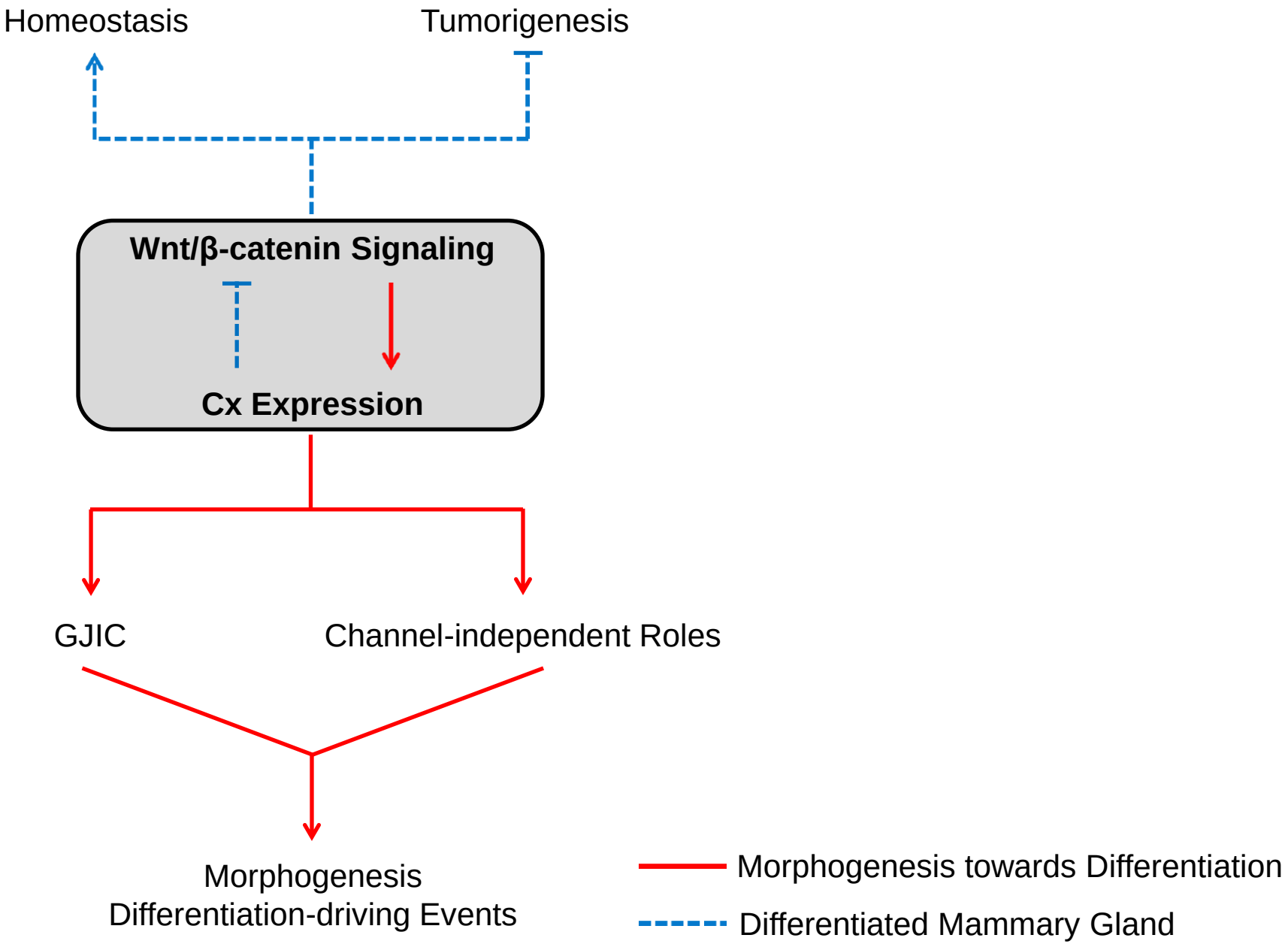
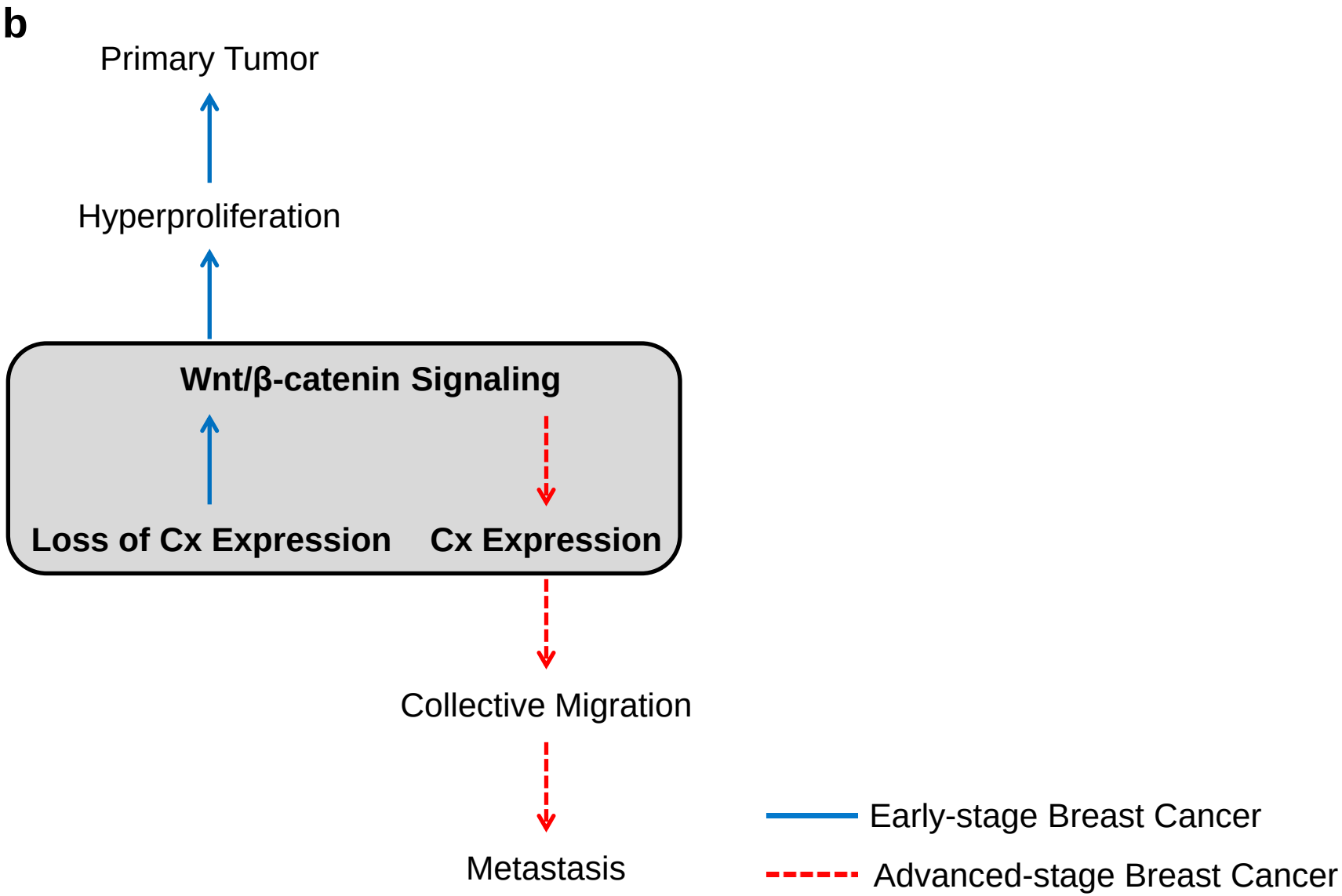
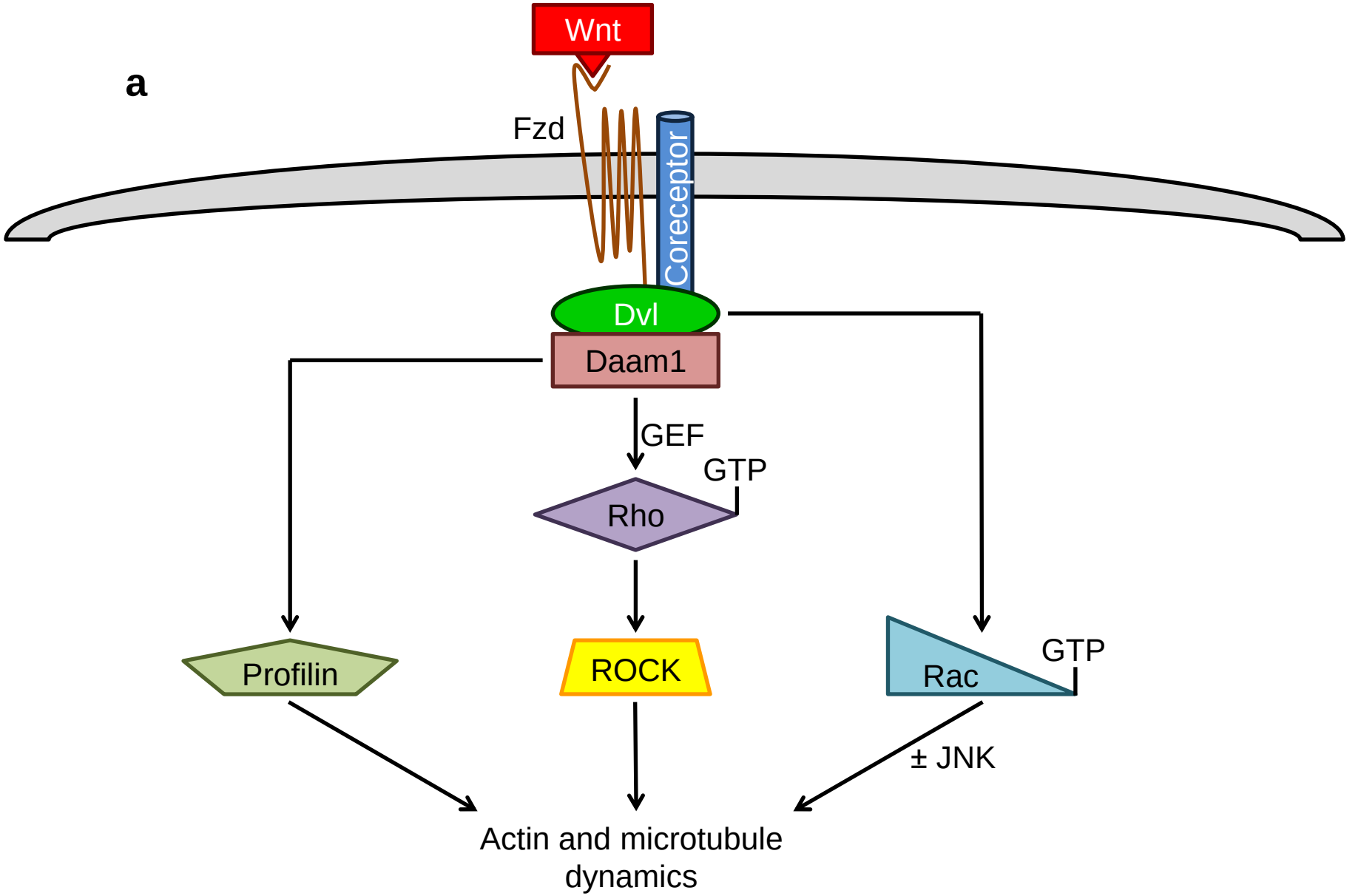
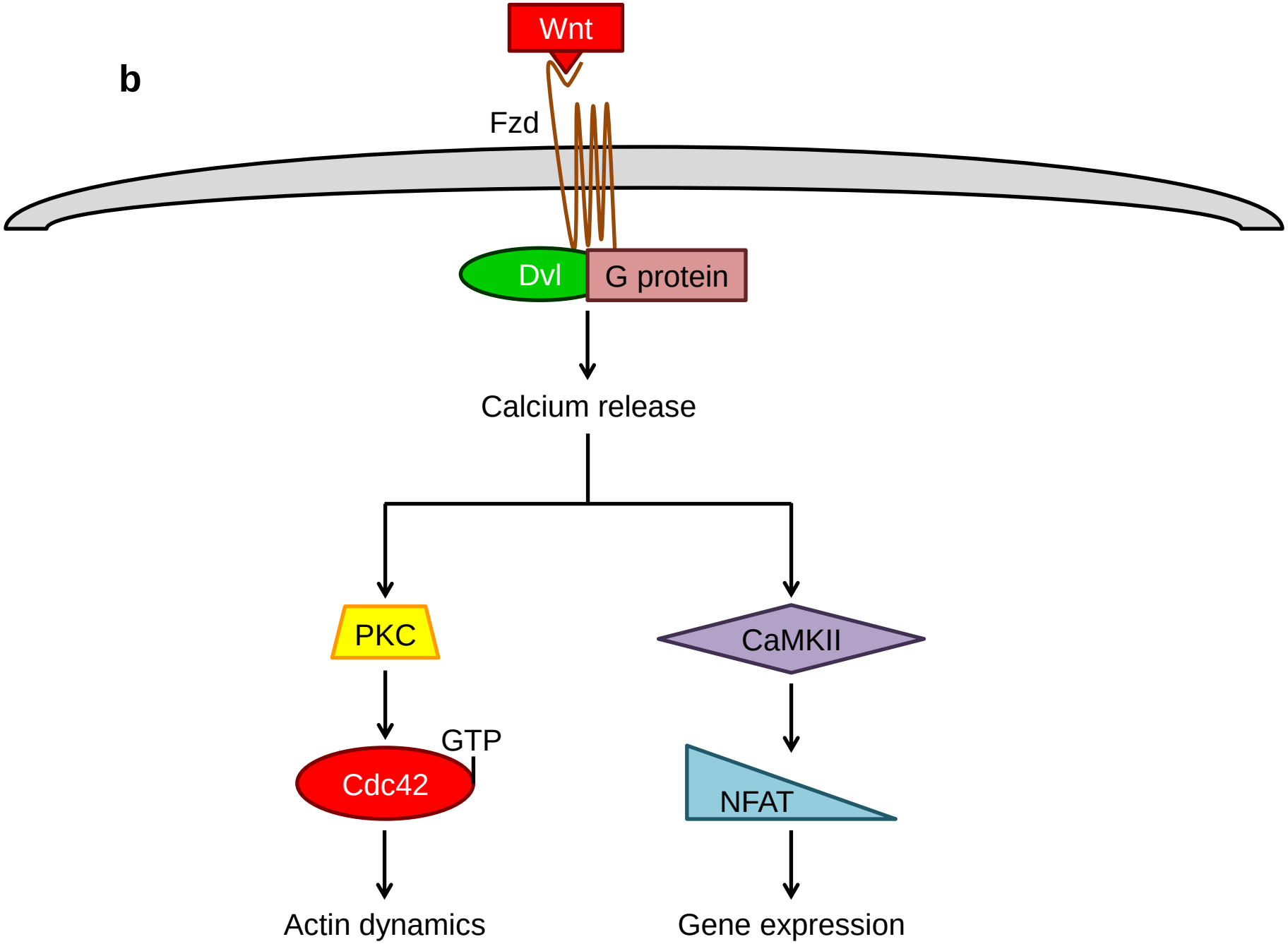
β-catenin
Gro/TLE
TCF/LEF
Transcription of Wnt/β-catenin target genes

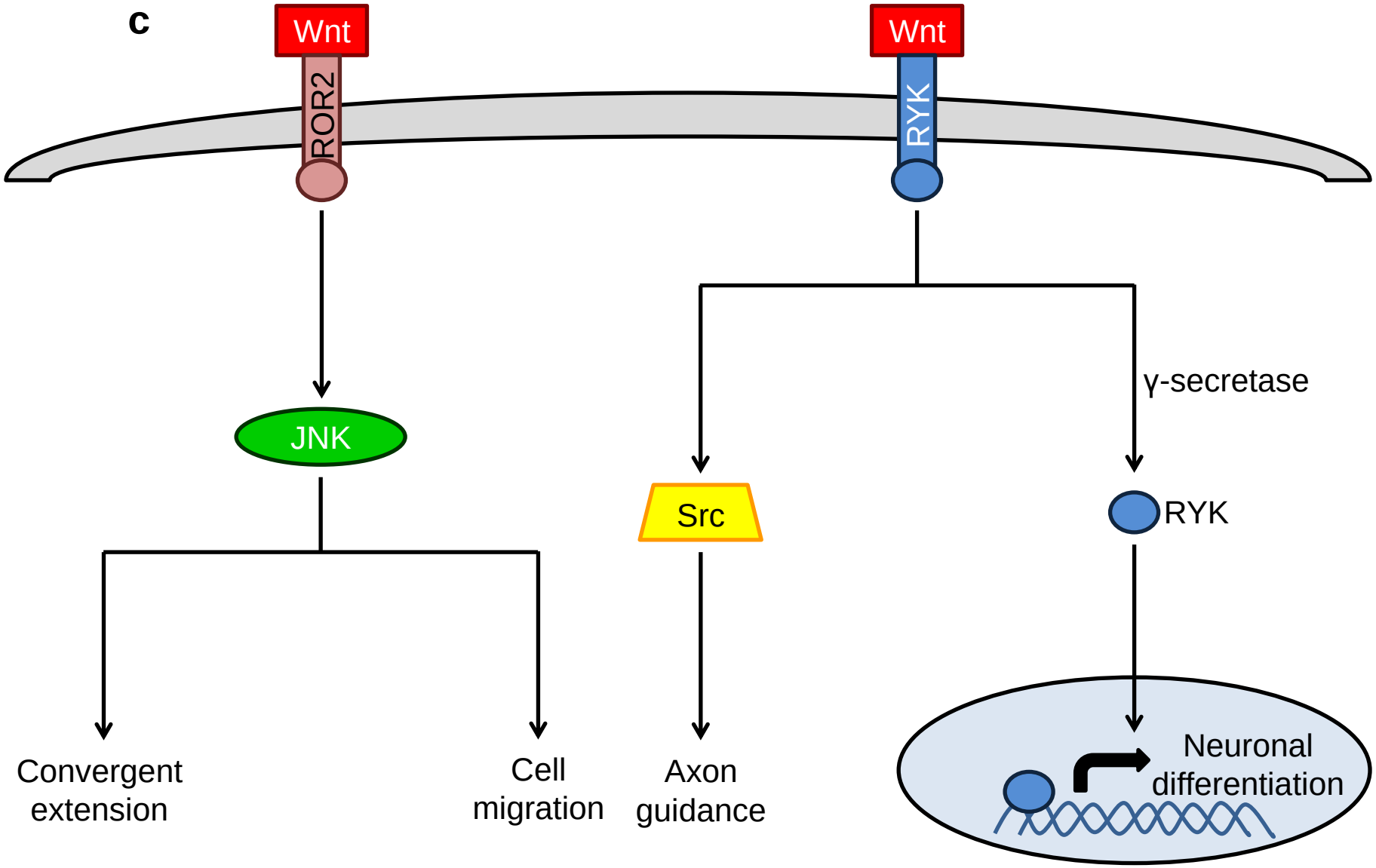
a


Homeostasis
Tumorigenesis
Wnt/β-catenin Signaling
Cx Expression
GJIC
Channel-independent Roles
Morphogenesis
Differentiation-driving Events
Morphogenesis towards Differentiation
Differentiated Mammary Gland


b
Primary Tumor
Hyperproliferation
Wnt/β-catenin Signaling
Loss of Cx Expression
Cx Expression
Collective Migration
Metastasis
Early-stage Breast Cancer
Advanced-stage Breast Cancer


a
Wnt
Fzd
Coreceptor
Dvl
Daam1
GEF
GTP
Rho
Profilin
ROCK
GTP
Rac
± JNK
Actin and microtubule dynamics


b
Wnt
Fzd
Dvl
G protein
Calcium release
PKC
CaMKII
GTP
Cdc42
NFAT
Actin dynamics
Gene expression


c
Wnt
ROR2
Wnt
RYK
JNK
Src
γ-secretase
RYK
Convergent extension
Cell migration
Axon guidance
Neuronal differentiation