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REVIEW

Developmental signaling pathways regulating mammary stem cells and contributing to the etiology of triple-negative breast cancer

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Abstract Cancer has been considered as temporal and spatial aberrations of normal development in tissues. Similarities between mammary embryonic development and cell transformation suggest that the underlying processes required for mammary gland development are also those perturbed during various stages of mammary tumorigenesis and breast cancer (BC) development. The master regulators of embryonic development Cripto-1, Notch/CSL, and Wnt/β-catenin play key roles in modulating mammary gland morphogenesis and cell fate specification in the embryo through fetal mammary stem cells (fMaSC) and in the adult organism particularly within the adult mammary stem cells (aMaSC), which determine mammary progenitor cell lineages that generate the basal/myoepithelial and luminal compartments of the adult mammary gland. Together with recognized transcription factors and embryonic stem cell markers, these embryonic regulatory molecules can be inappropriately augmented during tumorigenesis to support the tumor-initiating cell (TIC)/cancer stem cell (CSC) compartment, and the effects of their deregulation may contribute for the etiology of BC, in particular the most aggressive subtype of BC, triple-negative breast cancer (TNBC). This in depth review will present evidence of the involvement of Cripto-1, Notch/CSL, and Wnt/β-catenin in the normal mammary gland morphogenesis and tumorigenesis, from fMaSC/aMaSC regulation to TIC generation and maintenance in TNBC. Specific therapies for treating TNBC by targeting these embryonic pathways in TICs will be further discussed, providing new opportunities to destroy not only the bulk tumor, but also TICs that initiate and promote the metastatic spread and recurrence of this aggressive subtype of BC.

Keywords Cripto-1 · Notch/CSL · Wnt/β-catenin · fMaSC/aMaSC · TIC/CSC · TNBC

Introduction

The mammary gland is a very dynamic organ, which starts to develop in the embryo by signals exchanged between the ventral epithelial cells and underlying mesenchymal cells [1]. However, most of mammary gland developmental program completes postnatally, and the gland will become entirely functional only in adult life. During mouse embryonic development, specification of mammary epithelial cell fate occurs at mid-gestation as cells aggregate to form a bilateral milk line followed by the appearance of two sets of distinct placodes, one on each side of the embryo. The subsequent formation and ingrowth of mammary placode buds is followed by branching of the nascent mammary duct [2, 3]. A rudimentary ductal tree then forms during the latter stages of embryonic development via progressive elongation, canalization, and branching of the anlage (primitive mammary organ), which continues to invade the fat pad precursor. Ductal structures persist in a relatively quiescent state following birth until puberty. During mouse puberty, which occurs at 4–6 weeks of age, mammary epithelial cells undergo variable amounts of terminal end bud (TEB) formation, duct elongation,

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dichotomous, and lateral side branching. In the female human breast, terminal duct lobular unit formation and stromal expansion also occur during puberty [2]. At pregnancy, in both species, the mammary gland undergoes extensive side branching and alveolar development occurs in preparation for lactation. Upon cessation of lactation and during involution, the gland regresses to a previous state, which resembles the virgin or nulliparous gland. Epithelial cell plasticity of the developing mammary gland is influenced by ovarian (e.g., estrogen, progesterone) and pituitary (e.g., prolactin) hormones, as well as locally derived growth factors that are hormonally regulated and can be expressed either in the epithelial cells, stromal cells, adipocytes, mast cells, or macrophages that surround the TEBs [4]. The fundamental processes required for the inductive events in mammary bud development are also those that are perturbed during various stages of mammary tumorigenesis and breast cancer (BC) development [5]. Embryonic signaling pathways that regulate mammary cell fate specification and patterning in the embryo through fetal mammary stem cells (fMaSC), and in the adult organism through adult mammary stem cells (aMaSC), can also determine mammary progenitor cell lineages, which will generate the basal/myoepithelial and luminal compartments of the mammary gland (Fig. 1). The regulatory molecules that modulate these processes can be inappropriately augmented during tumorigenesis to support the tumor-initiating cells (TICs)/cancer stem cells (CSCs) compartment [5]. In this review, we will discuss the roles of the embryonic development master regulators Cripto-1, Notch/CSL, and Wnt/β-catenin in mediating mammary gland morphogenesis and cell fate specification, and the effects of their deregulation in the etiology of BC, in particular the most aggressive subtype of BC, triple-negative breast cancer (TNBC). Lastly, we will outline existing clinical applications using Cripto-1, Notch/CSL, and Wnt/β-catenin signaling molecules as therapeutic targets in TNBC.

Cripto-1, Notch/CSL, and Wnt/β-catenin signaling in Mammary Gland Development

The epidermal growth factor (EGF)-Cripto-1-FRL-1-Cryptic (CFC) family encompasses several members identified in deuterostomes, especially in vertebrates [6]. Human Cripto-1 is the founding member of this family and primarily functions during embryogenesis as a co-receptor for the transforming growth factor beta (TGF-β) family of ligands Nodal and growth and differentiation factors 1 and 3 (GDF-1, -3) in a canonical pathway, leading to the activation of the Activin type I (Alk4,7)/Activin type II (ActRII) receptor complex that subsequently triggers phosphorylation of Smad-2/Smad-3, and the activation of this Smad-dependent intracellular signaling pathway mediated by Smad-4 [7] (Fig. 2). Cripto-1 is anchored to the cell membrane by a glycosylphosphatidylinositol (GPI) lipid moiety within lipid rafts [8] and can also function in a Smad-independent non-canonical pathway, acting as a ligand for the GPI-anchored heparan sulfate proteoglycan Glypican-1, which is also tethered to the plasma membrane within lipid raft microdomains, to activate the tyrosine-protein kinase (c-Src)/mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3 K)/protein kinase B (AKT) signaling pathways that regulate cell proliferation, motility, and survival [7] (Fig. 2). Both canonical and non-canonical Cripto-1 signaling pathways can be significantly amplified by GRP78/BIP (78 kDa glucose-egulated protein/binding immunoglobulin protein), which belongs to the endoplasmic reticulum stress response pathway [9] (Fig. 2). During embryonic mammary gland development, Cripto-1 is expressed in the mesenchymal cells surrounding the mammary placodes but not in the epithelial placodes, similar to the expression pattern of Msx2, Lef1, and β-catenin in the canonical Wnt/β-catenin signaling pathway [4]. In fact, *Cripto-1* is a direct target gene in the Wnt/β-catenin pathway [10]. Postnatally, Cripto-1 can be detected at low levels in the ductal epithelial cells of the virgin mouse mammary gland and its expression significantly increases during early to mid-pregnancy, and early lactation, being also detected in human breast milk [11]. In the virgin mouse mammary gland, Cripto-1 is localized in the luminal epithelial cells and cap cells of the advancing TEBs and within the branching ducts, and contributes to the induction of epithelial plasticity, epithelial-to-mesenchymal transition (EMT), and ductal invasion into the mammary fat pad of the developing gland [4]. In the initial stages of pregnancy, Cripto-1 is upregulated by progesterone and can directly regulate progesterone receptor (PR) expression in luminal progenitor cells of the mouse mammary gland, triggering side branching, and alveologenesis induced by the receptor activator of nuclear factor kappa B (NF-κB)-ligand (RANKL) signaling pathway [12] (Fig. 1).

Mammals possess four Notch membrane-tethered receptors (Notch1-4) and five ligands (Jagged 1, 2 and Delta-like, Dll 1, 3, and 4) [13]. Notch receptors undergo proteolytic maturation by a furin-like protein convertase (S1 cleavage) in the Golgi/Endoplasmic Reticulum, and Notch signaling turns on upon ligand-receptor interaction in a short-range intercellular communication system between neighboring cells, which induces sequential proteolytic cleavages of the four mature Notch receptors. The second cleavage (S2) occurs in the extracellular domain

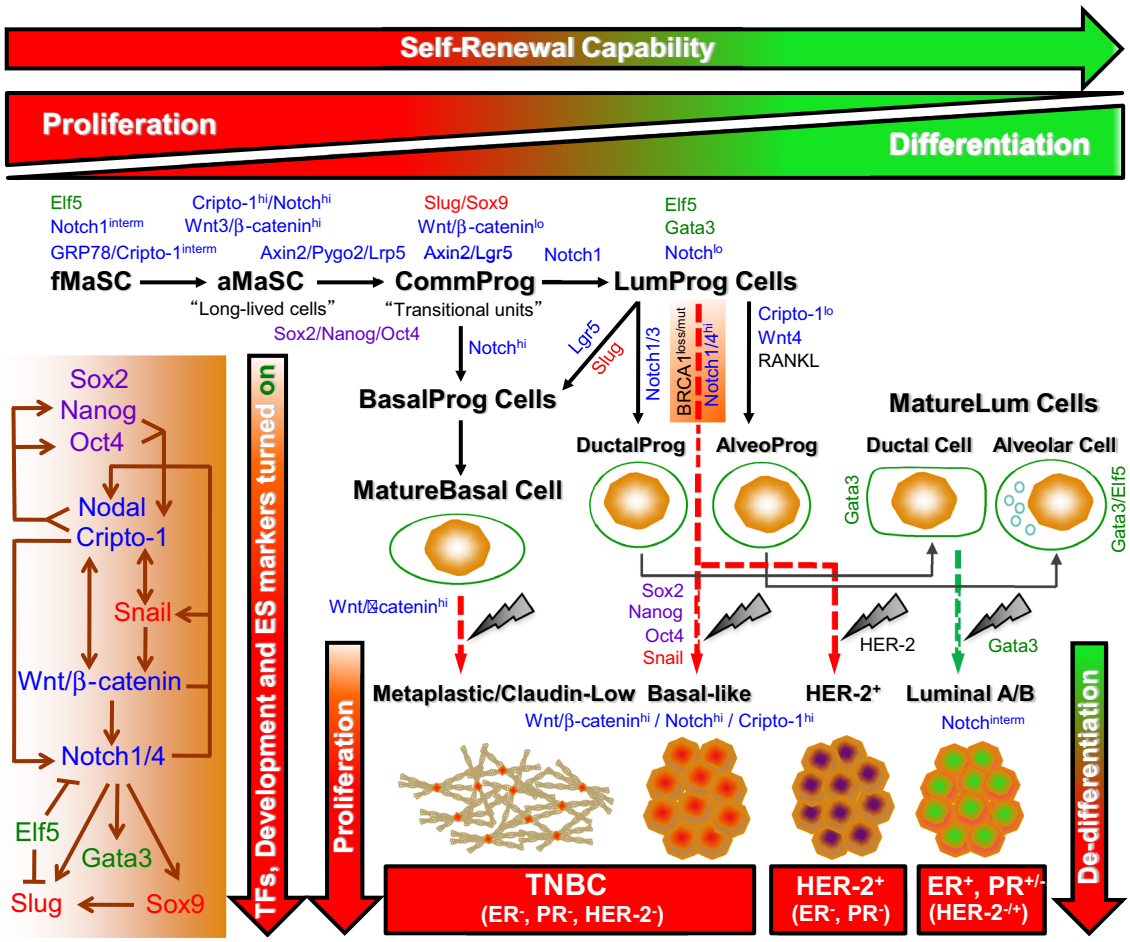

Fig. 1 Hypothetical hierarchical organization of normal mammary epithelial cells and their potential relationship with defined human breast cancer molecular subtypes. Isolation of cells from mouse and human mammary tissues supports this schematic hierarchical organization of the mammary gland development. Multipotent fetal mammary stem cells (fMaSC) give rise to adult mammary stem cells (aMaSC). Long-lived aMaSCs in turn generate transitional common progenitor cells (CommProg) that can commit to either basal/myoepithelial (BasalProg) or luminal progenitor (LumProg) cells. BasalProg cells develop mature basal cells (MatureBasal), and LumProg cells produce either ductal progenitor (DuctalProg) or alveolar progenitor cells (AlveoProg), which in turn generate mature luminal cells (MatureLum) in the form of mature ductal or alveolar cells, respectively. Disruption in the homeostasis of any of these cells—from the most primitive/undifferentiated (fMaSC/aMaSC) to the most differentiated cells (ductal and alveolar cells)—may lead to the development of mammary/breast tumors. The more undifferentiated, greater the potential of proliferating and self-renewing for multiple generations, and therefore accumulating deleterious genetic and/or epigenetic alterations that can persist over the lifespan of an individual. These cells may possibly become putative cancer stem cells (CSC) or tumor-initiating cells (TIC), implicated in the etiology of the distinct molecular subtypes of breast cancer (BC). For mammary/breast tumor development, reactivation of embryonic stem cell (ES) markers (shown in *purple*), certain transcriptional factors (TF) (shown in *red* or *green*) and crucial developmental signaling pathways (shown in *blue*), such as Cripto-1, Notch/CSL and Wnt/β-catenin, result in increased proliferation and restoration of self-renewal capacity of mammary epithelial cells, as well as enable the initiation of a de-differentiation program. A schematic illustration of recognized regulatory cross-talk occurring between master regulators of mammary gland development and tumorigenesis discussed in this review is depicted in figure. Some examples of commonly mutated and/or amplified genes other than *BRCA1/2* in the different subtypes of BC include *TP53*, *PTEN*, *AKT*, *RB1*, *PIK3CA*, *TBX3*, *FOXA1*, *CDH1*, *CBFB*, *MAP3K1*, *NF1*, *KRAS*, *KIT*, *MET*, *FGFR1/2*, *BRAF*, *EGFR*, *RUNX1*, *MLL3*, and *MAP2K4* [40], but they are not represented in figure. *TNBC* triple-negative breast cancer; *ER* estrogen receptor; *PR* progesterone receptor; *interm* intermediary; *hi* high; *lo* low; *mut* mutation

(ECD) and is mediated by metalloproteases of the A disintegrin and metalloproteinase (ADAM) family. The last cleavage (S3) happens within the transmembrane domain and is mediated by a gamma secretase presenilin complex, allowing the release and translocation of the intracellular domain of Notch (NICD) into the nucleus where it associates with a transcription complex containing a CSL (CBF-1 in human and RBPJ-κ in mouse) transcription regulator. This interaction converts the multiprotein CSL co-repressor complex into a transcription co-activator complex resulting in increased expression of Notch target genes, including the Hes and Hey family [13] (Fig. 2).

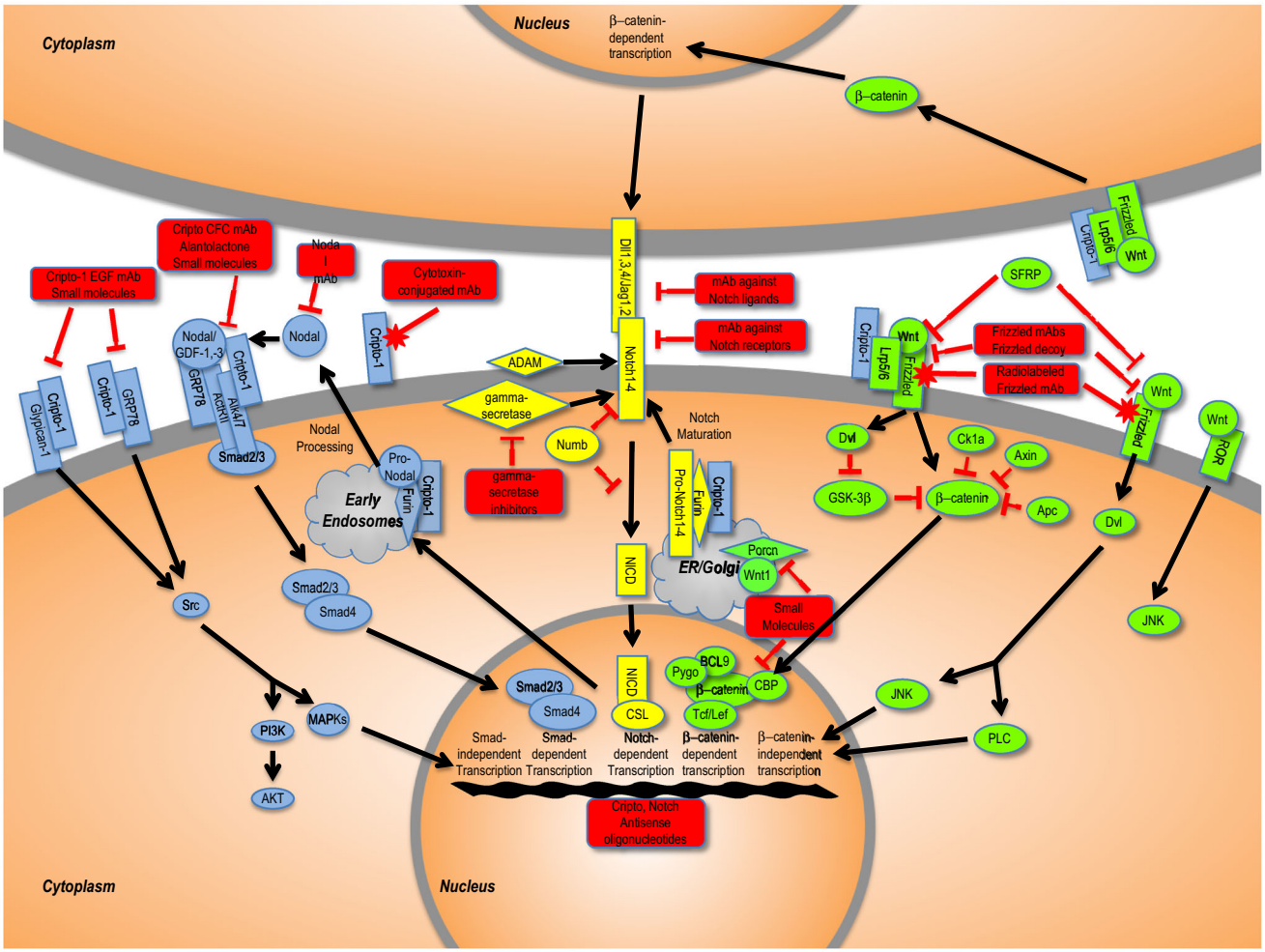

Fig. 2 Signaling cascades of Cripto-1, Notch/CSL, and Wnt/β-catenin. The transduction of Cripto-1 signaling (shown in *blue*) occurs through both the canonical signaling pathway mediated by Smad2/3 with Smad4 following Nodal/GDF-1, -3 activation of the Activin type I receptor (Alk4,7)/Activin type II (ActRII) receptor complex and the non-canonical pathway resulting in MAPK activation by GRP78 or Glypicans through Src. Therapeutic interventions (shown in *red*) for this pathway include antisense oligos to inhibit Cripto-1 expression, small molecule antagonists, and monoclonal antibodies (mAbs) against the EGF domain of Cripto-1 to inhibit the non-canonical pathway, Alantolactone, small molecule antagonists, mAbs against the CFC domain of Cripto-1 and the Cripto-1 ligand Nodal to inhibit the canonical Smad-mediated signaling, and a cytotoxin-conjugated non-neutralizing mAb against the amino terminus of Cripto-1 to directly kill target cells. Notch/CSL activation (shown in *yellow*) occurs when a Jagged (Jag) or Delta-like (Dll) ligand bound to an adjacent cell binds a Notch receptor on the target cell facilitating cleavage by ADAM proteases extracellularly followed by the gamma secretase complex intracellularly, releasing the Notch intracellular domain (NICD) which translocates to the nucleus to transactivate transcription through CSL. Therapeutic strategies against Notch include Notch antisense oligonucleotides, mAbs against Notch ligands, mAbs against Notch receptors, and gamma secretase inhibitors. The canonical Wnt/β-catenin pathway (in *green*) involves the binding of a Wnt ligand to a Frizzled receptor (Fzd) with the aid of a Lrp co-receptor. Stimulation of Fzd results in the activation of Disheveled (Dvl) which in turn inhibits the β-catenin destruction complex, glycogen synthase kinase 3 β (GSK-3β)/axin/casein kinase 1 a (Ck1a)/adenomatous polyposis coli (Apc), allowing β-catenin to translocate to the nucleus to initiate Tcf/Lef transactivation in the context of Pygo, BCL9, and CBP. The non-canonical Wnt pathway (in *green*) involves the activation of Fzd and Dvl leading to the subsequent activation of the c-Jun N-terminal kinase (JNK) and phospholipase C (PLC) triggering gene activation independently of β-catenin. The Wnt family can also activate the ROR family of receptor tyrosine kinases to potentiate JNK activation. Therapeutic strategies have involved mAbs against various members of the Frizzled family, a Frizzled 8-Fc-conjugated decoy receptor, a radiolabeled anti-Frizzled-10 mAb, and small molecule antagonists against Porcupine and β-catenin/CBP binding

Canonical CSL-mediated Notch signaling is crucial in the regulation of mammary cell communication during embryogenesis, stem cell self-renewal, cell lineage commitment, cellular proliferation, differentiation, and apoptosis in the mouse and human mammary gland [14, 15]. Notch expression is temporally and spatially regulated in mammary epithelial cells during mouse mammary gland development. In the postnatal mammary gland, Notch1-3 mRNA expression levels increase from 5 weeks of age through early pregnancy, decreasing at late pregnancy, and

displaying the lowest levels during early involution (apoptotic stage) and late involution (quiescent mammary gland) [16]. Notably, Notch3 mRNA expression is the most abundant at all the stages of mammary gland development, while Notch4 expression is almost undetectable [16]. Transgenic mice expressing a constitutively active Notch4 (Int3) under the control of the mouse mammary tumor virus (MMTV) promoter showed restriction of cell fate selection in the mammary epithelial cells, exhibiting arrested mammary gland development with reduced ductal growth and secretory lobule development, resulting in loss of lactation [17]. Eventually, these mice develop poorly differentiated adenocarcinomas [17]. However, mammary glands of mice harboring the Int3 transgene driven by the whey acidic protein (WAP) promoter, where activity is restricted to the secretory mammary epithelial cells, only showed disruption in lobular differentiation, but not in ductal growth/extension [18]. Interestingly, mammary ducts of transgenic virgin WAP-Int3 females were able to fill the fat pad completely and seemed morphologically normal, but during gestation at late pregnancy, lobular development was severely impaired with almost no alveolar outgrowth, which failed to exhibit milk protein production in full-term pregnant females. Female mice fail to lactate due to this severe impairment in alveolar development. Importantly, mammary tumorigenesis occurred in all breeding and non-breeding WAP-Int3 females, although with a higher latency period in nulliparous females [18].

The Wnt/β-catenin signaling pathway includes 19 Wnt ligands, 10 Frizzled (Fzd) receptors, and two co-receptors—low-density lipoprotein receptor-related protein (Lrp) 5 and 6. Binding of secreted Wnt ligands to Fzd receptors and Lrp5/6 co-receptors at the cell surface induces a complex series of interactions to activate the canonical Wnt/β-catenin signaling. Fzd, upon binding to a subset of Wnt ligands, recruits the mediator disheveled (Dvl) and interferes with the constitutively active β-catenin multiprotein destruction complex, composed of Axin/conducting, adenomatous polyposis coli (Apc), and casein kinase 1 a (Ck1a), which enhance glycogen synthase kinase 3 beta (GSK3β) phosphorylation and results in inactivation of GSK3β function. Normally, GSK3β phosphorylates β-catenin, marking it for destruction through an ubiquitin-mediated degradation pathway [13]. However, if GSK3β becomes phosphorylated by Ck1a, its ability to phosphorylate β-catenin will be thereby blocked, and then non-phosphorylated β-catenin will accumulate in the cytoplasm, consequently enhancing its translocation to the nucleus, where it can activate target genes, such as Cripto-1, Cyclin-D1, and Lgr5, upon interaction with T cell factor (Tcf)/Lymphoid enhancer factor (Lef) DNA binding proteins [10, 19, 20] (Fig. 2). Wnt/β-catenin signaling is essential for initiating formation of the mammary milk line in the embryo and further becomes restricted to mesenchymal cells surrounding the mammary epithelial placodes that invaginate to form flask-shaped buds [15]. The activation of β-catenin signaling in the embryonic mammary mesenchymal compartment can promote stem cell amplification and branching morphogenesis [21]. In the adult mammary gland, Wnt/β-catenin signaling works in a cell-specific manner, inducing ductal morphogenesis in basal epithelial cells in a paracrine manner, and promoting alveologenesis in hormone receptor-negative luminal epithelial cells [22]. Remarkably, Wnt4, which is inducible by progesterone, regulates an important hormonal phase in the luminal progenitor mammary epithelial cell compartment, activating progesterone-induced side branching and RANKL effectors during lobulo-alveolar development in a paracrine fashion [23] (Fig. 1). In fact, mammary glands lacking the expression of Wnt4 exhibited delayed alveologenesis during pregnancy [24]. On the other hand, mammary epithelial cells overexpressing Wnt4 and Wnt1 develop hyperplastic outgrowths, and with Wnt1 subsequent mammary tumors [25, 26]. Interestingly, ectopic expression of Wnt3 in mammary epithelial cells expressing Cripto-1 significantly enhanced cell transformation induced by Cripto-1 [10]. Conversely, Cripto-1 can enhance Wnt/β-catenin signaling activation by directly binding to the Lpr5 and Lpr6 co-receptors and facilitating Wnt3 binding to these co-receptors [10]. In addition to the potential role in inducing mammary tumorigenesis, a positive feedback-signaling loop may exist between these two pathways that might be also essential in regulating mammary alveologenesis, considering that both Cripto-1 [12] and Wnt/β-catenin [23] are essential for RANKL-induced alveologenesis. A comprehensive summary of the expression and function of Wnt/β-catenin signaling effectors during mammary gland development can be found in Alexander and colleagues [25].

Cripto-1, Notch/CSL, and Wnt/β-catenin signaling in fetal mammary stem cells (fMaSC) and adult mammary stem cells (aMaSC)

fMaSC and aMaSC

The observation that the mammary gland exhibits plasticity and a unique regenerative capacity through multiple cycles of pregnancy, lactation, and involution, accompanied by dynamic changes in proliferation, differentiation, cell death, and tissue remodeling, suggests that there exist subpopulations of renewable stem and/or progenitor cells underlying these processes. A population of epithelial cells exists in the fetal mammary anlage of the embryo, which has a self-renewal capacity and the ability to generate daughter cells that can differentiate into distinct progenitor cell lineages that will form all of the cell types that are

present in the mature mammary gland [5, 27] (Fig. 1). The cell specification process is initiated by the expansion of fMaSCs, which constitute the earliest MaSC population [27]. fMaSCs are bipotent ($CK8^+/CK14^+$) and can be enriched using the cell surface marker profile $CD24^{hi}/CD49f^{hi}$ [27]. Although differing from the $CD29^{hi}/CD24^+$ aMaSC marker signature, fMaSCs are also able to reconstitute fully differentiated mammary outgrowths with functional and morphologically normal structures upon transplantation into the cleared mammary fat pad, with a repopulation capacity about four-fold higher as compared to the repopulation frequency of the aMaSCs [27]. Moreover, fMaSCs present significantly higher clonogenic capability as compared to aMaSCs [28]. However, aMaSCs are likely to derive from their embryonic counterpart fMaSCs, and along with their potential of extensive self-renewing for multiple generations, both populations are thought to harbor inherent tumorigenic potential as subsequent mammary development proceeds and continues postnatally. Kenney and colleagues showed that aMaSCs can survive up to 6–7 divisions and may exist either as long-lived (non-dividing) cells or as proliferating-differentiating cells (transitional units) [29]. A lineage tracing study reported that aMaSCs are unipotent [30]. Conversely, a more recent study provides strong evidences for the existence of a bipotent aMaSC population, which gives raise to both basal and luminal cells, and coordinates ductal homeostasis and remodeling of the adult mammary gland [31]. Upon asymmetrical division, aMaSC subpopulations can organize the mammary epithelium into two distinct amplifying cell lineages that are usually defined by their clonogenic activity in vitro, which are either basal/myoepithelial or luminal progenitors. Basal/myoepithelial cell lineages express p63 [32], smooth muscle actin and basal cytokeratins (CK5 and CK14) and are localized in a basal position adjacent to the basement membrane, presenting contractile properties [1]. The luminal lineage is governed by the expression of steroid hormone receptors and luminal cytokeratin proteins (CK8, CK18, and CK19), as well as β3 integrin (CD61), which subdivides the total luminal population into committed luminal progenitors ($CD29^{lo}/CD24^+/CD61^+$) and mature luminal cells ($CD29^{lo}/CD24^+/CD61^-$) [33]. The mature luminal cells are represented by the ductal sub lineage, which will form the luminal structures, and the alveolar sub lineage, which will generate the milk-producing alveolar units from alveolar progenitors arising from the luminal progenitor compartment [1].

Additionally, several key transcriptional regulators are involved in the determination of the aMaSC population and also in the initiation and maintenance of luminal progenitor cells (Fig. 1). For instance, the Sox9 transcription factor directly regulates the Slug promoter [34] and together they modulate aMaSC activity [35]. In fact, primary mammary epithelial cells containing Sox9 and Slug knocked-down exhibited a significantly reduced aMaSC-enriched basal subpopulation [35]. Conversely, their co-expression in differentiated luminal cells is sufficient to convert these cells into aMaSCs with long-term reconstituting ability in vivo [35] (Fig. 1). Interestingly, overexpression of Slug alone in luminal progenitor cells can convert these cells into a basal state [35]. In addition, the lack of Slug expression in the mouse mammary gland delays mammary gland development during puberty, and epithelial cells isolated from these glands overexpress the estrogen receptor (ER) and the transcription factor Gata3, and loose their potential to generate secondary mammospheres [36]. Gata3 is a necessary master regulator for enhancing and maintaining cell fate specification of luminal progenitor cells into mature ductal cells during puberty and induces the differentiation of luminal progenitor cells into mature alveolar cells during pregnancy [33] (Fig. 1). Notably, ectopic expression of Gata3 in the aMaSC subpopulation induces milk protein expression in the absence of any hormonal lactogenic stimuli [33]. *Elf5* encodes a member of the ETS family of transcription factors and is upregulated in fMaSCs, as well as regulates alveolar cell differentiation of mammary cells during pregnancy [37]. Importantly, Elf5 directly represses the transcription of Slug, impairing a basal-fate program, and the lack of Elf5 during pregnancy and lactation activates an EMT-like phenotype [38]. Besides differentiation, Elf5 is also essential for morphogenesis of mature alveolar cells, with no influence on ductal cells [39]. Moreover, Elf5 might prevent the de-differentiation program of luminal progenitor cells into a more primitive state, since its loss increases the repopulating capacity of aMaSCs [38] and activates Notch/CSL signaling pathway [39] (Fig. 1).

The long-term survival of aMaSCs could allow more time for cumulative genetic lesions in multiple genes (e.g., *Tp53, PTEN*) to be acquired as DNA damage/mutations may increase throughout postnatal development [40]. Progenitor cells adversely affected by these genetic alterations and also epigenetic modifications, such as altered methylation of genes could then persist over the lifespan of an individual and effectively create a quiescent subpopulation of cells that may well harbor potentially deleterious mutations, instabilities, and genomic translocations. These cells could then become activated by altered extrinsic signaling pathways and convert these quiescent cells into putative TICs, fostering unlimited, or extended self-renewal capacity since human breast tumors may take many years to arise [5]. Importantly, during tumorigenesis, luminal progenitor cells can acquire the ability to interconvert between phenotypes, which could potentially explain the cellular heterogeneity within the different types of BC. The luminal progenitor compartment can become very metaplastic upon oncogenic stress, wherein luminal

progenitor cells in particular can produce basal-like tumor cells [41] especially after excessive Notch/CSL signaling activation [42] and loss and/or mutation of the tumor-suppressor gene *BRCA1* [43, 44] (Fig. 1).

Cripto-1, Notch/CSL, and Wnt/β-catenin regulate fMaSC and aMaSC

Cripto-1 is a direct downstream target gene of the pluripotency embryonic stem (ES) cell master regulators Nanog and Oct-4 [45], and reciprocally, in cooperation with Nodal and Activin, Cripto-1 is essential in maintaining Nanog and Oct-4 expression through a Smad-dependent signaling pathway [46]. Nanog, Oct4 and Sox2 are present in aMaSCs and luminal progenitor cells population, and their expression decreases when these cells start to differentiate [47, 48] (Fig. 1). Likewise, Spike and colleagues (2014) found that Cripto-1 could promote pluripotency of fMaSCs and aMaSCs ex vivo and enhance their potential to reconstitute the mammary gland through an aMaSCs/progenitor cell subpopulation (Fig. 1). In addition, they demonstrated that the cell surface receptor GRP78/BIP is required for fMaSC and aMaSC activity and for Cripto-1 responsiveness [49]. Together, Cripto-1 and GRP78/BIP seem to play a developmentally conserved role in regulating the fMaSC and aMaSC phenotypes.

Notch/CSL signaling can also regulate aMaSCs to promote self-renewal and enhance lineage-specific commitment of basal/myoepithelial progenitor cells, as well as to increase their proliferation rate, with no apparent effects on fully differentiated mammary epithelial cells [50] (Fig. 1). Stimulation of the canonical Notch pathway also promotes branching morphogenesis in three-dimensional matrigel cultures, which can be completely inhibited by a Notch4 blocking antibody or a gamma secretase inhibitor, that prevents S3 cleavage and processing of the Notch receptors [50]. Notch3 activation directly stimulates commitment of luminal progenitor cells to a luminal cell fate commitment [51]. In fact, Notch3 is expressed in a highly clonogenic luminal progenitor population that gives rise to ductal lineages. These cells are able to survive multiple successive pregnancies, since Notch3 possibly restricts their proliferation and clonal expansion [52]. Notch1 was also found to commit aMaSC expansion to the luminal cell fate, being preferentially activated in the ductal luminal epithelium in vivo [53], and inappropriate Notch1 activation promotes self-renewal and transformation of luminal progenitor cells [53]. Recently, Rodilla and colleagues showed that Notch1 is expressed in the fMaSC population, which possesses a very restricted lineage potential to exclusively generate an ER^- luminal lineage postnatally, and alveolar progenitors that express Notch1 and expand during pregnancy, surviving multiple successive involutions [54].

The Wnt/β-catenin signaling component Axin2 has been studied in the mouse mammary gland, where Axin2-expressing mammary cells were found to reside exclusively in the aMaSC-enriched subpopulation [55]. Remarkably, van Amerongen and colleagues showed that Axin2-responsive mammary cells resident in the basal compartment of prepubescent mammary gland are unipotent and retain long-term proliferative potential. In fact, Axin2-positive cells transplanted into cleared mouse mammary fat pads after puberty generated only basal cells [56]. However, these Axin2 basal-restricted cells switched to bipotent stem cells during pregnancy, as they gave rise to both luminal and basal cells present in the alveolar structures [56]. Lgr5 is another Wnt/β-catenin member that marks a rare subpopulation of cells within the basal compartment and induces mammary reconstitution activity in vivo [57]. Interestingly, a single Lgr5-expressing cell can efficiently generate an entire functional mammary outgrowth [57]. During early postnatal development, a very small fraction of Lgr5-positive mammary cells was able to switch from luminal-committed cells to a basal-fate phenotype [58] (Fig. 1). Also, Wnt3a can clonally expand aMaSCs for multiple generations and enhance their ability to generate functional mammary glands following in vivo transplantation [55]. Additionally, luminal cells with decreased levels of Wnt4 inhibited the self-renewing capacity of aMaSCs, as shown by the enrichment of differentiation genes and a significant reduction of proliferation-associated genes [53]. The Wnt/β-catenin co-receptor Lrp5 also plays crucial roles in maintaining aMaSC activity in the mammary gland [59]. Importantly, Cripto-1 binds to Lrp5 and facilitates Wnt3a binding to Lrp5, enhancing Wnt/β-catenin signaling through cytoplasmic stabilization of β-catenin and elevated β-catenin/Tcf transcriptional activation [10]. Likewise, Wnt3a increases migration, invasion, and anchorage-independent growth of Cripto-1-expressing normal mouse mammary epithelial cells [10]. The Wnt/β-catenin signaling co-activator Pygopus (Pygo) 2 was shown to suppress luminal differentiation of the aMaSC-enriched population by coordinating the activity of Wnt/β-catenin and Notch signaling pathways [60]. Interestingly, in parous mice, the expression of Notch/CSL pathway members seems to be increased in the aMaSC-enriched population in comparison to the expression of Wnt/β-catenin target genes [61].

Cripto-1, Notch/CSL, and Wnt/β-catenin signaling pathways are enhanced in breast cancer (BC)

Distinct subtypes of BC may originate from different cells of origin [62]. As previously discussed, abnormal regulation of the multifaceted mammary epithelial hierarchy may further give rise to different BC molecular subtypes,

possibly explaining the heterogeneous nature of human BC [63]. There are currently 5 defined molecular subtypes of BC: claudin-low/metaplastic, basal-like, HER-2-positive (HER-2^+), luminal A, and luminal B [64] (Fig. 1). Basal-like and claudin-low breast cancers are often referred as TNBC, since the tumors are typically negative for ER, PR, and HER-2 ($ER^-/PR^-/HER\text{-}2^-$) and are usually associated with a poor prognosis and decreased survival rate [65].

While Cripto-1 is required for mammary gland morphogenesis and hematopoietic stem cell renewal, unregulated expression of Cripto-1 is found in a number of different types of human cancers, including breast, prostate, cervix, colon, bladder, lung, and melanoma [7]. Additionally, soluble Cripto-1 levels are elevated in the plasma obtained from colon, lung, glioblastoma, and BC patients, what suggests that Cripto-1 levels in human plasma or serum may have potential clinical diagnostic and prognostic significance [7]. A genome-wide association study for Cripto-1 serum levels performed in an isolated human population, aiming to identify genetic variants associated with the levels of circulating Cripto-1 protein in the serum, confirmed its fundamental role in embryonic development and cancer [66]. Moreover, GRP78/BIP is expressed on the surface of tumor cells [67] where it preferentially binds to GPI-anchored proteins [68], and it has been shown that Cripto-1/GRP78/BIP complex increases tumor growth [69]. Cripto-1 is also involved in the reprogramming of differentiated BC cells into TICs through the induction of an EMT program [4]. Recently, it has been shown that the transcription factor Snail, which is a mediator of EMT, can regulate the expression of Cripto-1 [70] and vice versa [71] (Fig. 1), controlling responses such as cell motility, transformation, and differentiation. Additionally, Cripto-1 has a strong angiogenic activity in cultured endothelial cells and is involved in regulating new blood vessel formation in developing breast tumors [7]. Furthermore, Cripto-1 may be involved in the immune escape of malignant cells and immunosuppression of tumor cells by enhancing macrophage phagocytic activity and upregulating the production of anti- and pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), through the NF-κB signaling pathway [72]. Moreover, Cripto-1 modulates Notch/CSL and Wnt/β-catenin signaling pathways, which are also involved in the etiology of BCs, as next discussed. Cripto-1 can function as a chaperone for Notch receptors in the Golgi/Endoplasmic Reticulum compartment by enhancing S1 cleavage of Notch receptors by recruiting furin-like proteases and sensitizing S1 cleavage and ligand-induced activation of Notch/CSL signaling [73]. Additionally, Cripto-1 acts as an auxiliary positive modulator by facilitating the binding of Wnt3 to the Lrp5 and Lrp6 Wnt co-receptors, thereby enhancing Wnt/β-catenin signaling [10].

Constitutive activation of Notch signaling is present in approximately 50 % of BCs. Recently, a pathway and network analysis of gene expression and immunohistochemical profiling of BC has revealed that Notch signaling is critical for maintaining $ER^+/PR^+/HER\text{-}2^-$ breast tumors and for cell cycle regulation in $ER^+/PR^+/HER\text{-}2^+$ breast tumors [74]. Interestingly, deregulation of Notch signaling appears to occur early in pre-invasive ductal carcinomas. Elevated expression levels of Notch1, Notch4, and Hes1 can be detected in ductal carcinoma in situ (DCIS) as compared to normal mammary tissue [75]. Several mutations have been observed in the Notch1/2 receptors in TNBC [76]. Furthermore, exome sequencing continues to reveal mutations in Notch signaling components within BC, among them multiple gain-of-function and frequent loss or inactivation of the negative regulator Numb [75]. Notably, Notch signaling is implicated in regulating tumor-stroma interactions, metastasis and chemotherapeutic resistance in BC. Both Notch1 and Notch4 receptors are overexpressed in basal-like TNBC and vascular endothelial cells with a subcellular localization different from that in hormone-positive luminal BC [77]. In addition, Notch1 directly induces the vascular endothelial growth factor receptor 3 (VEGFR-3) in blood vessel endothelial cells, which regulates vascular development and subsequently lymphangiogenesis [77]. Meta-analysis of microarray data from over 4000 BC patients has revealed that the elevated Notch/CSL pathway activity is independently associated with an increased rate of disease recurrence [78]. Likewise, in mice, Notch signaling remains activated in a subset of dormant residual tumor cells that persist following HER-2 down-regulation and accelerates tumor recurrence [78]. Additionally, a Notch1-Slug axis was shown to be vital for the Jagged1-induced promotion of EMT, migration, and invasion of BC [79].

Canonical Wnt/β-catenin signaling can be constitutively activated in different types of cancer, such as colorectal carcinoma, by a variety of mechanisms including mutations in β-catenin, Apc, and Axin [75]. In general, these mutations enable β-catenin to escape destruction and drive oncogenic Wnt/β-catenin signaling. However, mutations in Apc or β-catenin generally occur in low frequency in BC. Despite the lack of direct gene mutations, elevated levels of β-catenin expression have been detected in approximately 60 % of BCs, and additionally in many cases, aberrant hypermethylation of the Apc gene promoter has also been reported, as well as upregulation of Wnt/β-catenin signaling pathway target genes such as Lef1 and Axin2, especially in the TICs population of BC [75]. Aristizabal-Pachon and collaborators recently described genetic alterations in Axin2, which were associated with a higher risk of developing BC, and also an aberration in the β-catenin destruction complex expression profile associated with BC.

Moreover, the same study reported for the first time expression of the β-catenin destruction complex in circulating tumor cells from peripheral blood of BC patients [80]. Secretion of Wnt/β-catenin ligands into the niche microenvironment may have a non-cell autonomous/paracrine effect on cells at the invasive edge of breast tumors by increasing tumor cell proliferation, EMT, and subsequent tumor cell invasion. Analysis of human cancer datasets confirms that elevated Wnt/β-catenin gene expression in BC correlates with expression of genes with an EMT signature, such as Vimentin, Snail, and Twist [81]. Several novel molecular mechanisms that might contribute to aberrant Wnt/β-catenin activation and progression in BC have been described. Among them, BCL9, a nuclear co-factor that binds β-catenin and Pygo, modulates canonical Wnt/β-catenin signaling by promoting β-catenin-mediated transcription [82]. The TCGA breast cancer database indicates that BCL9 is significantly amplified in basal-like subtypes of BC. Furthermore BCL9 expression was also detected in $ER^-/PR^-/HER\text{-}2^+$ DCIS lesions [82]. Also, the Pitx2 isoform 1 transcription factor, which is a target gene in the Nodal/Cripto-1 and Wnt/β-catenin signaling pathways, is known to contribute to the invasion of BC cells. Pitx2 was found in the bone marrow of BC patients and was significantly associated with metastatic BC development [83].

Cripto-1, Notch/CSL, and Wnt/β-catenin signaling pathways regulate tumor-initiating cells (TIC) and contribute to the etiology of triple-negative breast cancer (TNBC)

Similar to fMaSC, aMaSC, and luminal progenitor cells, a small subpopulation of TICs or CSCs exists, which share characteristics with the above normal mammary stem cells and possess self-renewal and partial differentiation capacity in human breast tumors [1]. TICs are proposed to recapitulate the original phenotype of BC, to enhance tumor relapse and contribute to chemotherapy and radiotherapy resistance and/or facilitate metastasis [84]. Interestingly, the invasion and metastasis program of TNBC is intrinsically related to an aMaSC population signature, which predisposes this subpopulation of BC cells to facilitate subsequent metastasis [85]. However, molecular profiling studies have revealed that the more primitive aMaSC/basal signature is most closely aligned with metaplastic claudin-low subtype of BC [65, 86], suggesting that a very aggressive aMaSC subpopulation may give rise to claudin-low BCs (Fig. 1). Conversely, the expression profile of basal-like BC is not concordant with the aMaSC-enriched subset, but with the expression signature of a luminal progenitor population [87]. In fact, Pfefferle and colleagues showed that 94 % of basal-like tumors exhibited a luminal progenitor profile, while claudin-low subtype represented the highest percentage of primitive aMaSCs (18 %) [88], indicating indeed that they may arise from a more primitive aMaSC population, and most probably due to aberrations in Wnt/β-catenin signaling [62] (Fig. 1). Intriguingly, 59 % of the claudin-low tumors were also classified as presenting luminal progenitor characteristics [88].

The processes of somatic cell reprogramming, TICs establishment and metastatic colonization are dependent on transitions between EMT and its reverse process mesenchymal-epithelial transition (MET) that occurs in the colonized end organs following metastasis, such as in the lung and brain [89]. Similarities between embryonic development and cell transformation during oncogenesis have been previously reported [90]. In particular, EMT performs an essential role during embryonic development in gastrulation [4]. However, if EMT is inappropriately activated in BC cells, it can induce the formation of migratory mesenchymal cells that possess stem cell-like phenotypes, TICs properties, and an invasive capacity with a high metastatic potential [90, 91]. These similarities have led to the identification of common ES cell markers, such as Nanog, Oct4, and Sox2, as also expressed in mammary tumors [92–94], significantly increasing the potential of TICs cells to form mammospheres and promote metastatic invasion [47]. Additionally, a Nanog/Oct4/Sox2 expression signature was directly associated with high-grade TNBC basal-like subtype and with poor clinical outcome [95] (Fig. 1). Notably, the embryonic signaling pathways addressed in this review, Cripto-1, Notch/CSL and Wnt/β-catenin, when inappropriately reactivated in populations of aMaSC and/or progenitor cells, may generate TICs, which can potentially drive mammary cell transformation, tumor progression, and invasion in adult mammary tissues (Fig. 1).

Cripto-1 is also one of the stemness genes that have been shown to generate induced pluripotent stem cells (iPSC) and is essential in maintaining normal ES cells and initiating EMT, as well as being expressed in TIC subpopulations and contributing to early cancer progression [84, 96–98]. In fact, Cripto-1 expression is significantly enhanced in basal-like and $HER\text{-}2^+$ breast tumors (Fig. 1), which derive from a common luminal progenitor population, suggesting that Cripto-1 might be important in the etiology or progression of BC subpopulations [99]. In this context, Cripto-1 has been suggested as a novel therapeutic target in a Notch4-driven spontaneous model of TNBC [100]. Cripto-1 promoter activity was detected in spindle-like areas exhibiting EMT phenotype of mouse primary tumors that resemble human TNBC, but not in lung metastasis. Additionally, Cripto-1 knockout resulted in a significant inhibition of primary tumor growth and a pronounced reduction in the number and size of pulmonary metastases, suggesting that Cripto-1 may contribute to mammary oncogenesis in TNBC [100].

Elevated expression of the Notch3 receptor has been correlated to TNBC [52]. Recently, a novel Notch reporter system revealed that all Notch receptors (1-4) are able to mediate the regulation of BC TICs, and specifically Notch4 expression was correlated with poor patient survival in a TCGA breast tumor dataset analysis (cBioPortal) [101]. Indeed, both Notch1 and Notch4 are expressed in aMaSCs and TICs, but in particular, Notch4 has been shown to maintain the human TICs population [14, 102]. Treatment with the gamma secretase inhibitor RO4929097 decreased primary tumor growth and significantly reduced the number of metastatic lung nodules in an animal model that resembles human TNBC and overexpresses Notch4 [100]. Additionally, the activity of TICs and Notch4 was proven to govern both *de novo* and acquired tamoxifen resistance, and Notch4 inhibition reduced TICs activity in patient-derived xenograft (PDX) tumors with acquired anti-estrogen resistance [103]. Therefore, the combination of endocrine therapy and Notch4-targeting therapy might overcome anti-estrogen resistance in human BC.

Wnt/β-catenin directs cell fate determination at various stages of development and is implicated in EMT during early embryogenesis, such as in gastrulation. In adults, Wnt/β-catenin signaling has a key role in the regulation of tissue stem self-renewal, particularly in the intestinal crypts, hair follicles, bone growth plates, bone marrow, mammary gland morphogenesis, and in mouse and human mammary tumorigenesis [15, 89]. Accumulating evidence indicates a critical role of Wnt/β-catenin signaling in the functioning of TICs. A recent report has shown that blockade of Wnt/β-catenin signaling suppressed BC metastasis by inhibiting TICs-like phenotype [104]. Moreover, another study showed that pan-PI3K inhibitors, a treatment option for TNBC, activated canonical Wnt/β-catenin signaling through stimulation of Wnt ligand secretion leading to treatment resistance in TNBC cells [105]. This resistance was decreased through combined treatment with pan-PI3 K and Wnt inhibitors [105]. These findings indicate that Wnt/β-catenin pathway activation conferred resistance in TNBC. Collectively, these findings suggest a crucial role and potentially functional cross-talk of Cripto-1, Wnt/β-catenin, and Notch/CSL in the regulation of TICs, EMT, and resistance to therapy in TNBC.

Cripto-1, Notch/CSL, and Wnt/β-catenin signaling members as new prognostic tools and therapeutic targets for tumor-initiating cells (TIC) in triple-negative breast cancer (TNBC)

Due to the reactivation of several developmental signaling pathways in the initiation and progression of breast tumors in concordance with their respective roles in TICs and TNBC, they may well be appropriate candidates as prognostic markers and therapeutic targets. The prognostic value of all three developmental signaling pathways has been explored to varying degrees in several cancers. The determination of which pathways are activated in TICs or progenitor subpopulations could lead to patient-specific therapy that targets TICs involved in tumor initiation, metastasis, and chemotherapeutic and radiation resistance. Since Cripto-1 can promote mammary tumorigenesis, EMT [4], and can be also present in a TICs population [84], its expression might provide be a significant prognostic marker. In fact, a previous study from our laboratory found Cripto-1 expression at least 10-fold higher in the plasma of breast and colon cancer patients compared to healthy control subjects [106]. Likewise, Cripto-1 is expressed at significant levels in high-grade breast tumors and correlates with an overall decrease in patient survival [7, 107]. Similar findings have been found in gastric cancer patients with a concurrent down-regulation of E-cadherin, signifying an increase of metastatic disease [108]. Recently, our laboratory has found the expression of anti-Cripto-1 autoantibodies in patients with glioblastoma [109]. The production of autoantibodies against a target such as Cripto-1 could provide a predictive marker, as well as a potential therapeutic strategy as some autoantibodies act as agonistic ligands [109]. To date, therapies directed against Cripto-1 have mostly been non-clinical and employed a variety of approaches including antisense oligonucleotides, humanized mouse, or rat monoclonal antibodies, either conjugated or not to drugs, and a naturally occurring small natural product, Alantolactone [7] (Fig. 2). Recently, a study examining a panel of small molecules from the NCI diversity library found 4 compounds that were able to inhibit canonical Cripto-1 signaling through Smad2 and varying degrees of efficacy in inhibiting non-canonical signaling through the c-Src/AKT/MAPK pathway [109] (Fig. 2). Furthermore, a phase I clinical trial of refractory solid tumors using a humanized monoclonal antibody against Cripto-1 conjugated with the cytotoxin maytansinoid complex DM4 (BIIB015) was completed and showed no toxicity with preliminary partial positive responses [110, 111] (Fig. 2). In addition, when BIIB015 was used in combination with paclitaxel for treating human xenograft mouse mammary tumors from the TNBC cell line MDA-MB-231, this dual treatment protocol conferred a robust activity by inhibiting tumor growth by 90 % in comparison to treatment with either of the agents alone, suggesting that BIIB015 could enhance the efficacy of chemotherapeutic agents against TNBC [111]. Most recently, Cripto-1 DNA vaccination generated protective response elicited by $CD8^{+}$T cells against lung metastasis in a highly metastatic melanoma mouse model by targeting a small subpopulation of melanoma stem cells expressing Cripto-1 [112]. This

DNA vaccine was also highly effective against aggressive TICs (listed as an abstract), which makes it very attractive to be tested in a TNBC model. Further clinical testing will be necessary to elucidate the true value of these approaches and other drug and/or antibody combinations.

As with Cripto-1, members of the Notch/CSL pathway have been assessed for potential prognostic value. High expression levels of Notch ligands and receptors have been associated with poor patient survival [75]. The mRNA expression of Notch1 receptor was correlated with increased mortality in PR^- tumors, while augmented expression of Notch2, 3, and 4 mRNA correlated with better overall survival for all BC patients [113]. Another study, examining the expression of the Notch family members by in situ hybridization revealed that the expression of Notch1, Notch3, and Jagged1 predicted higher mortality and increased patient relapse [114]. Although the sample size for this study was small, tumors expressing high levels of Notch1, Notch3, or Jagged1 were phenotypically classified as TNBC. To this point, patients with tumors co-expressing high levels of both Notch1 and Jagged1 had a stronger correlation with mortality [114]. To date, there have been a number of approaches approved for clinical trials to target the Notch/CSL pathway in multiple tumor types. For example, there have been a number of clinical trials using gamma secretase inhibitors either alone or in combination with chemotherapeutic agents to treat solid tumors as wells as leukemias [115] (Fig. 2). Monoclonal antibodies against various Notch signaling components are also in clinical trial, such as antibodies directed against Notch1, Notch2/3, and DII4 [115] (Fig. 2). Other approaches such as siRNAs that are directed against Notch1, Notch3, or CBF-1 showed that the $CD44^+$ subset of MCF-7 breast tumor cells underwent apoptosis and were further sensitized to radiotherapy in vitro [116].

Due to the pivotal role of the Wnt/β-catenin pathway in TNBC, the possibility exists to use the expression of proteins of this signaling pathway as prognostic indicators in TNBC, as well as therapeutic targets. Interestingly, the expression of Wnt1 is higher in BC tissues as compared to normal breast tissues, but there is no difference observed in the clinical grade of the tumors suggesting that Wnt1 might be an early marker of tumorigenesis, but not a significant prognostic marker [117]. High expression of the tyrosine kinase-like orphan receptor 1 (ROR1), loss of the Wnt signaling antagonist Frizzled-related protein 1 (SFRP1) in early breast tumors, and nuclear localization of β-catenin all correlate with increased metastasis and reduced overall survival in BC patients in general with higher correlations observed in basal-like and claudin-low breast tumors [118–120]. Interestingly, a study of ovarian cancer detected autoantibodies against a number of proteins, including β-catenin [121]. In contrast, another study found that the expression of Apc, a negative regulator of β-catenin activity, is increased in grade-3 BC tumor tissue while showing a decrease in the normal surrounding breast tissue. In this study, β-catenin was found in the cytoplasm of tumor cells but did not localize to the nucleus and its expression decreased as the tumor grade increased [117]. Overall, the expression levels of certain Wnt/β-catenin family members correlate with a more aggressive tumor type and therefore a poor outcome. Current clinical strategies for targeting the Wnt/β-catenin pathway include small molecule antagonists against CBP/β-catenin binding and Porcupine (LGK-974), the latter being involved in Wnt secretion [115] (Fig. 2). Therapies targeting the Fzd proteins include a monoclonal antibody directed against Fzd-1/2/5/7/8 (Vantictumab), a radiolabelled monoclonal antibody for Fzd-10, and a Fzd-8-Fc decoy fusion protein (Fig. 2). In particular, Vantictumab in combination with paclitaxel and LGK-974 are currently in Phase I clinical trials for luminal BC and TNBC, respectively [115] (Fig. 2). Other experimental approaches to target the Wnt/β-catenin pathway that have been explored, but are beyond the scope of this review can be found in Anastas and Zheng and colleagues [122, 123]. In summary, these three families of signaling pathways are potentially attractive targets for specific therapies designed to attack breast tumors, in particular TNBC. Furthermore, the use of combinatorial cocktails of chemotherapeutic agents with these signaling inhibitors in conjunction with radiotherapy provides the opportunity to destroy not only the bulk tumor, but also TICs that initiate and promote the metastatic spread and recurrence of TNBC.

Conclusion

In this comprehensive review, we present clear evidence of the involvement of Cripto-1, Notch/CSL, and Wnt/β-catenin signaling pathways in the fetal and adult mammary gland development through the modulation of fMaSC, aMaSC, and mammary progenitor cells. We also discuss the contribution of these signaling pathways in the generation of TICs and their involvement in the development, maintenance, and resistance to therapy of TNBC, and lastly, we provide information on new promising possibilities for the treatment of this aggressive subtype of BC.

Compliance with ethical standards

Conflicts of Interest All the authors declare no conflicts of interest.

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