Breast Cancer Stem Cells: Something Out of Notching?

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Abstract

We and others have established that the developmental Notch receptor signaling pathway is active in breast cancer cell lines, as well as in preinvasive and invasive primary samples. Recently, a role for Notch in regulating the hierarchy of stem and progenitor cells in both normal and cancer epithelium has been elucidated. Because inhibiting the Notch receptor signaling pathway is a possible future breast cancer therapy, here, we review the expression and activity of the different ligands and receptors and summarize the various ways in which the pathway's activity can be inhibited, and the likely effects of inhibition on different tumor cell subpopulations.

Introduction

The developmental Notch pathway is a juxtacrine cell-cell signaling mediator comprising five ligands in the sending cell that bind to any of four receptors present on the signal-receiving cell. The pathway is aberrantly activated in breast cancer cell lines as well as in preinvasive and invasive primary samples, although unlike T-cell leukemia, mutation of Notch receptor genes is not common. Notch seems to play a biological role in regulating the cellular hierarchy in both normal and cancer epithelium. In a recent article published in Cancer Research, we report that breast cancer stem cell (CSC) activity is governed specifically through Notch4 receptor signaling. Inhibiting the Notch signaling pathway is a possible future therapy, the success of which will depend on expression and activity of the pathway in different molecular subtypes of breast cancer. In addition, its activity can be modulated in multifaceted ways, which may determine the balance of breast tumor stem- and progenitor-cell activity.

Complexities of Ligand and Notch Receptor Expression and Activity

Notch signaling plays a major role in normal breast development and has been implicated in cancer initiation and progression. The pathway is made up of four transmembrane receptors (Notch1–4) and five transmembrane ligands \( \delta \)-like (DLL)1, 3, and 4, and Jagged1 and 2. Ligands expressed on the signaling cell bind to the extracellular domain of Notch receptors on an adjacent signal-receiving cell. This binding causes the intracellular domain (ICD) to be released following two sequential cleavages involving the A disintegrin and metalloproteinase 10 or 17 (ADAM10/17) and gamma secretase enzymes. Notch-ICD translocates to the nucleus where it interacts with the transcriptional mediator RBPJ/CSL and MAML as a complex that activates transcription of downstream target genes (Fig. 1). The Notch signaling pathway is known to regulate breast stem cell self-renewal and cell-fate determination, and the individual receptors seem to play distinct roles. Little is known about differential signaling downstream of different ligand and/or receptor combinations or whether each ICD has specific downstream targets. Some evidence exists that individual Notch receptors preferentially bind to specific targets via RBPJ/CSL independent, noncanonical signaling. For example, N4-ICD interacts with Smad, NFκB, and HIF1α proteins, and N1-ICD interacts with Slug (2–4). It is thought that, as well as possible differential targets, the distribution of individual receptors and ligands may play an important role in the regulation of Notch signaling. For example, Notch 1 and 3 are known to be expressed in the luminal cells within the normal breast (1, 4), whereas Notch 4 is somewhat restricted to the myoepithelial and/or basal compartment (1, 4). RBPJ knock-out and/or knock-down experiments depict an increased myoepithelial and/or basal compartment and suggest that Notch1 activation can direct cellular differentiation along the luminal lineage (5, 6). The Notch 4 receptor is expressed in the stem cells and/or bipotent progenitor cells in the normal breast, and inhibition of this receptor has been shown to reduce stem cell activity (7, 8). Activation of signaling with DLL1 ligand has also been shown to bias cell fate toward a myoepithelial and/or basal cell fate (8). Furthermore, a recent report demonstrates that the level of pathway activation can direct cell phenotype. Low Notch signaling results in normal breast cell hyperproliferation, whereas high levels of Notch signaling activity led to growth arrest (9).
Aberrant Notch Receptor Activity in Breast Cancer

A role for Notch in breast cancer was suggested two decades ago in viral mutagenesis experiments that revealed frequent insertion of mouse mammary tumor virus (MMTV) in the Int3/Notch4 locus in mouse mammary tumors. The mutagenic insertion led to a truncated and constitutively active form of the Notch4 receptor (10). Viral insertions in Notch1 resulting in a truncated and active protein also lead to mammary tumor formation or maintain tumors in MMTV/neu transgenic mice (11, 12).

High levels of ligands, receptors, downstream targets, and downregulation of Numb have also been reported in invasive breast cancer (1, 13–15). Recent evidence suggests that each receptor and ligand may play distinct roles in the development and progression of different tumor types. Overexpression of Notch4, for example, has been suggested as a candidate to classify triple-negative tumors (16), and the ligands Delta4 and Jagged1 have been shown to have differential effects on angiogenesis and bone metastasis (17). Notch3 has been shown to play a role in the survival and proliferation of HER2-negative tumors, and inhibition of this receptor causes growth cessation and increased apoptosis (18). Furthermore, Notch3 has been implicated in the survival and self-renewal of CSC in hypoxic environments that develop in large, more advanced tumors (19).

Aberrantly activated Notch signaling has been shown to play a causal role in the transformation of many different cancer types (20). In a few cases, Notch signaling has been shown to increase proliferation (for example, through the induction of CyclinD1 expression in kidney cancer cells; ref. 21), but in most cases, as in breast cancer, it seems to be through the regulation of apoptosis (20). Consequently, it is

Figure 1. A simplified schematic of Notch signaling depicting the multifaceted targeting of the pathway. The “sending cell” presents one of the three DLL or two Jagged (JAG) ligands, which binds to the extracellular domain of a Notch receptor (N1, 2, 3, or 4) on the “receiving cell.” The Notch ICD is released following two sequential cleavages by ADAM10/17 and gamma secretase enzymes. ICD translocates into the nucleus where it interacts with the transcriptional mediator RBPJ/CSL and the coactivator MAML1, thereby activating downstream genes. The pathway can be targeted in one of several ways: (1) gamma secretase inhibitor inhibiting the cleavage of the ICD from Notch receptors (1–4; refs. 1, 15, 16); (2) anti-DLL4 antibodies (18); (3) monoclonal antibodies to the ADAM10/17 cleavage site of Notch1/2/3 (19); and (4) α-helical peptides targeting MAML1 coactivators (20).
not surprising to find that elevated Notch signaling contributes to the resistance of breast cancer cells to chemotherapeutic drugs such as doxorubicin and docetaxel (22); recent mechanistic work suggests that this resistance is through the activation of Akt signaling via a Notch-induced autocrine factor (23). Aberrant Notch signaling also seems to contribute to the acquired resistance to Her2-targeted therapies (24), radiotherapy (25), and endocrine therapies such as tamoxifen (26). Consequently, it seems that Notch signaling contributes widely to treatment resistance.

Multifaceted Targeting of the Notch Signaling Pathway

The postbinding cleavage of the ICD is partly catalyzed by gamma secretase, which cleaves within the receptor transmembrane. Gamma secretase inhibitors (GSI) have been developed to block signaling by stopping the release of the ICD and, therefore, abrogating transcription of downstream targets (Fig. 1). GSIs have been used in vitro to inhibit breast CSC activity and in vivo to inhibit tumor growth and are currently being evaluated for use in breast cancer treatment (27). In preclinical studies, GSIs combined with other treatments have proved successful in resensitizing breast cancer cells to Her2-targeted (24), endocrine (26), chemotherapeutic (23), and radio- (25) therapies. However, GS1 treatment may only be successful in a subgroup of breast cancers because a gene signature of predicted response has been identified (28). This gene signature may prove useful for selection of a target patient population, which may aid in the design of clinical trials testing Notch inhibitors. In our study, we found two different GSIs were successful in delaying tumor formation, but that they did not affect all Notch receptors equally. These particular GSIs (DAPT and DBZ) did not inhibit mammosphere or tumor formation in vitro or in vivo as successfully as specific inhibition of Notch4 (1). It is not clear whether higher concentrations of GSIs or improved stability would be more effective. These findings suggest that more targeted inhibitors of the pathway could prove more successful. Furthermore, the Notch2 receptor has shown protective effects in breast cancer. Tumors that express this protein at high levels often have better prognosis and patient survival (29). Thus, inhibiting all Notch receptors indiscriminately may not be appropriate, and a clinical benefit may be found in more specific targeting of pathway components.

Anti-DLL4 antibodies have been developed that cause decrease tumor formation and growth (30). To reduce the complications of nonselective targeting of the pathway, many researchers are investigating antibodies that specifically target individual Notch receptors. Monoclonal antibodies have been identified that mask the ADAM10 cleavage domain in the Notch1, Notch2, and Notch3 proteins and specifically inhibit signaling through these receptors (31, 32). This work suggests that targeted antibody therapy may be possible for all four receptors, although none has been produced as yet to Notch4. Recently, direct inhibition of Notch signaling has been successfully demonstrated by blocking the protein-protein interaction of the ICD and CSL protein (33). Synthetic, cell-permeable stabilized α-helical peptides that target the coactivator Mastermind-like 1 (MAML1) were shown to decrease Notch signaling and to have effects very similar to those of GSIs. This research is extremely promising and offers a possible targeted Notch therapy. One possible drawback to this approach is that Notch4 has been shown in mouse mammary tumors to signal via both the canonical pathway and a noncanonical pathway that does not involve RBPJ (3). The significance in human tumors is unknown but could imply that targeting the MAML1 protein would not completely abrogate Notch4 signaling.

Concluding Remarks

Targeting Notch signaling as a treatment for breast cancer is promising, but as the pathway is active in other tissues it will be important to assess any toxicities associated with inhibition. If we are to properly understand the role of Notch signaling in breast cancer and the survival and expansion of breast CSCs, it is important that we study each receptor and ligand and their specific interactions more closely. Considering signaling as merely "the Notch pathway" may be too simplistic; we must consider the complexities of the pathway and learn more about it in order to develop successful Notch inhibition strategies for breast cancer prevention and treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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