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Mini-review

Notch signaling in breast cancer: From pathway analysis to therapy

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ABSTRACT

The Notch signaling pathway, which is highly conserved from sea urchins to humans, plays an important role in cell-differentiation, survival, proliferation, stem-cell renewal, and determining cell fate during development and morphogenesis. It is well established that signaling pathways are dysregulated in a wide-range of diseases, including human malignancies. Studies suggest that the dysregulation of the Notch pathway contributes to carcinogenesis, cancer stem cell renewal, angiogenesis, and chemo-resistance. Elevated levels of Notch receptors and ligands have been associated with cancer-progression and poor survival. Furthermore, the Notch signaling pathway regulates the transcriptional activity of key target genes through crosstalk with several other signaling pathways. Indeed, increasing evidence suggests that the Notch signaling pathway may serve as a therapeutic target for the treatment of several cancers, including breast cancer. Researchers have demonstrated the anti-tumor properties of Notch inhibitors in various cancer types. Currently, Notch inhibitors are being evaluated for anticancer efficacy in a number of clinical-trials. However, because there are multiple Notch receptors that can exhibit either oncogenic or tumor-suppressing roles in various cells, it is important that the Notch inhibitors are specific to particular receptors that are tumorigenic in nature. This review critically evaluates existing Notch inhibitory drugs and strategies and summarizes the previous discoveries, current understandings, and recent developments in support of Notch receptors as therapeutic targets in breast cancer.

1. Introduction

Breast cancer (BC) is the second leading cause of cancer deaths among women worldwide. In 2019, approximately 268,600 new cases of invasive BC and 62,930 new cases of *in situ* BC are estimated to be diagnosed, along with 41,760 BC-related deaths, in the U.S. alone. The majority of BCs are estrogen receptor-positive (ER^{+ve}) and can be treated using anti-hormonal therapy; however, recurrence is frequently observed in BC patients after five years of endocrine therapy. The dysregulation of several signaling pathways, including Notch, contributes to cancer progression and recurrence. Cross-talk between estradiol and Notch signaling has a major role in human breast carcinogenesis and angiogenesis [1–4]. In fact, recent studies have established that Notch signaling is dysregulated in multiple cancer types. Notch signaling contributes significantly to cell survival, proliferation, differentiation, apoptosis, tissue patterning, cell-fate decision, and morphogenesis [2]. Therefore, the Notch pathway might serve as a promising target for the treatment of BC. For example, cleavage of Notch receptors in the cytoplasm by γ-secretase is a major step in their activation, and inhibition of γ-secretase arrests the signaling pathway [3,4]. Recent studies suggest that γ-secretase inhibitors (GSIs) could be promising therapeutic agents for the treatment of cancers [5]. However, Notch receptors can act as either tumor suppressors or oncogenes, depending upon the cell context. Therefore, Notch inhibitors must be context-specific. In the present review, we summarize the established knowledge, as well as recent advancements, regarding the Notch signaling pathway in BC and evaluate the potential of its inhibition as a therapeutic approach for BC treatment.

2. Structure of Notch receptors

Notch genes, which are highly conserved from sea urchins to humans, encode transmembrane receptors. Initially, Notch receptors were identified as responsible for a specific “notch” shaped phenotype on the wings of Drosophila melanogaster [1,2]. In mammals, there is one ortholog (Notch 1) of the single Notch receptor in Drosophila; however, there are three additional mammalian Notch receptors (Notch 2–4), as well. Notch receptors consist of three domains: an extracellular domain

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Abbreviations

ANK	ankyrin repeats
BC	breast cancer
CSC	cancer stem-like cells
DLL	Delta-like ligand
EGF	epidermal growth factor
EMT	epithelial-to-mesenchymal transition
GSI	γ-secretase inhibitors
JAG	jagged
mAbs	monoclonal antibodies
MMTV	mouse mammary tumor virus
NECD	Notch extracellular domain
NICD	Notch intracellular domain
NLS	nuclear localization signal
NTM	Notch transmembrane domain
PEST	proline glutamic acid serine and threonine
RAM	RBP-jk association molecule
TAD	trans-activation domain

(NECD), a transmembrane domain (NTM), and an intracellular domain (NICD) [3]. The NECDs of Notch 1 and Notch 2 consist of 36 repeats of epidermal growth factor (EGF)-like repeats, which are required for ligand interactions, whereas the NECDs of Notch 3 and Notch 4 contain 34 and 29 EGF-like repeats, respectively [4,6–8]. The EGF- like repeats are followed by a negative regulatory region (NRR), which consists of cysteine-rich Lin12 (N/Lin12) repeats that modulate the interactions between the NECD and the membrane-bound NICD [9,10]. The Lin12 repeats prevent metalloprotease-driven, ligand-independent cleavage to stabilize the interactions between the subunits [11,12]. The NICD also includes an RBP-jk association molecule (RAM) domain, followed by seven ankyrin (ANK) repeats, two nuclear localization signals (NLSs), a trans-activation domain (TAD), which ends with a polyglutamine region (OPA), and a PEST sequence rich in proline (P), glutamic acid (E), serine (S), and threonine (T) residues [13–16]. The multiple phosphorylation sites present in the C-terminal region of the PEST sequence are responsible for the stability of NIC and consecutively trigger its ubiquitination [16–18] (Fig. 1). The NTM, which consists of a short extracellular region with a pair of highly conserved cysteine residues, mainly participates in heterodimerization [13,14].

3. Maturation of Notch receptors

The Notch precursor protein is fucosylated through its interaction with O- fucosyltransferase 1 (POFUT1 in mammals) in the endoplasmic reticulum [19–21]. The fucosylated protein is then transported and subjected to proteolytic cleavage by a Furin-like convertase at site 1 (S1) in the Golgi complex [22]. Finally, the Notch precursor is glycosylated by the Fringe family of N-acetyl-glucosaminidyl transferases, which add N-acetylglucosamine to O-linked fucose on the EGF-like repeats [23]. This matured Notch receptor gets transported on to the cell surface as a heterodimer (Fig. 2).

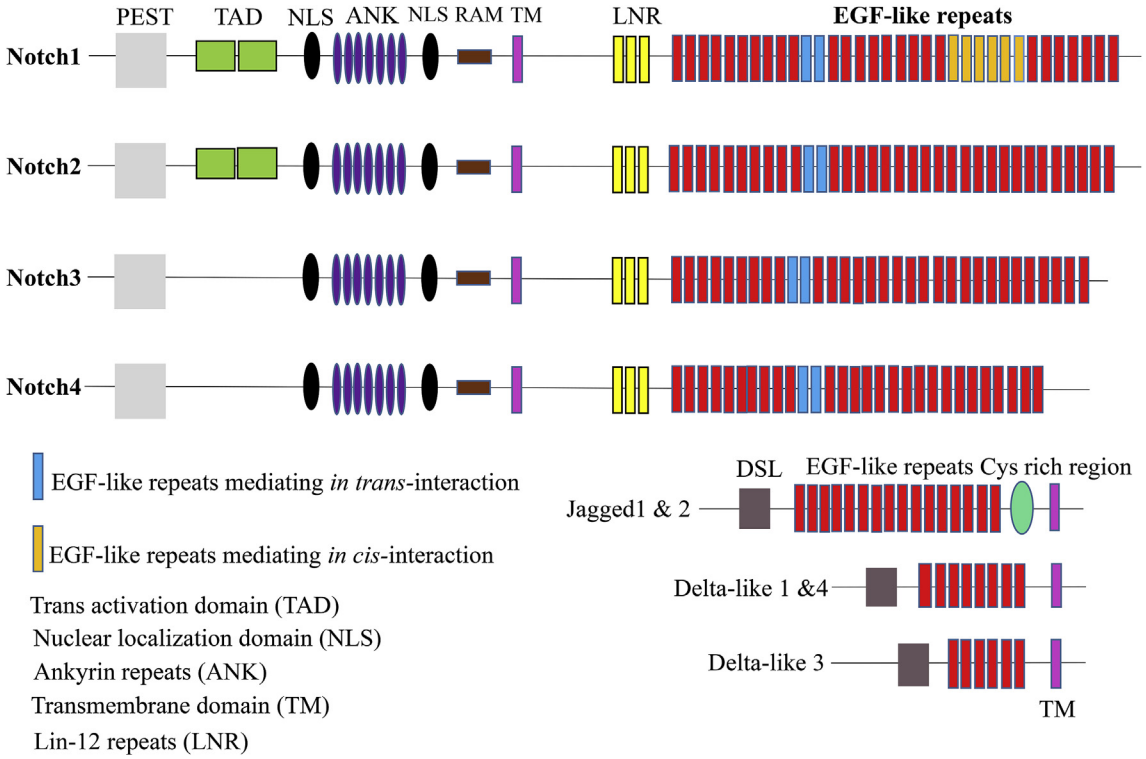

Fig. 1. Structure of Notch receptors and ligands.
Notch proteins are a highly conserved family of transmembrane receptors. Notch receptors and ligands contain multiple domains. The extracellular domains (NECDs) of Notch receptors 1–4 and their ligands (Jagged 1, Jagged 2, Dll1, Dll3, and Dll4) contain EGF-like repeats. Notch 1 and Notch 2 contain 36 EGF-like repeats, whereas Notch 3 and Notch 4 contain 34 and 29, respectively. The intracellular domains (NICDs) of Notch 1 and 2 contains a RAM (RBP-jk association molecule) domain, NLSs (Nuclear localization signals), an ANK (Ankyrin repeat) domain, a TAD (Trans-activation domain), and a PEST domain. The NICDs of Notch 3 and Notch 4 are similar, but the TAD is absent in both. The extracellular domain of Serrate-like ligands Jagged 1 and Jagged 2 consists of a DSL domain, EGF-like repeats, and a Cys-rich region. The extracellular domain of the Delta-like ligands (Dll1, Dll3, and Dll4) is similar, but the Cys-rich region is absent.

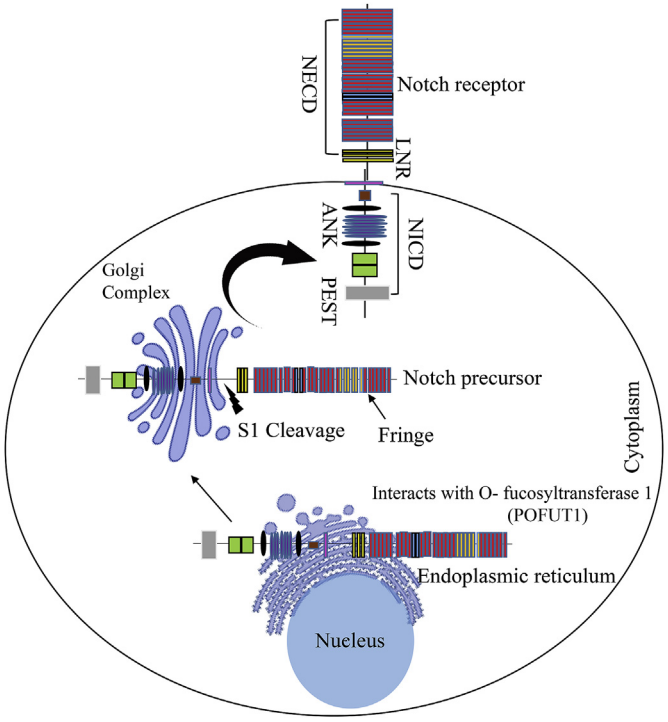

Fig. 2. Maturation of Notch receptors.
Notch receptors mature in the endoplasmic reticulum (ER) and Golgi complex. Fucosylation is essential and occurs through the interaction between the Notch precursor protein and O-fucosyltransferase 1 (OFUT1 in Drosophila, POFUT1 in mammals) in the ER. The fucosylated Notch precursor is then transported to the Golgi complex, where proteolytic cleavage by Furin-like convertase at site 1 (S1) occurs. Finally, the matured Notch is transported to the cell surface.

4. Activation of Notch receptors

4.1. Canonical pathway

In addition to the four Notch receptors, five canonical ligands have been identified in mammals, including humans: Delta-like ligand 1 (Dll1), Dll3, Dll4, and Serrate-like ligands jagged 1 and 2 [2]. The Notch receptors and ligands are type I cell surface proteins, and cell-cell interactions are instrumental for the activation of the Notch signaling pathway [24,25]. Activation of Notch receptor is mediated by a sequence of proteolytic events. A trans-interaction between a Notch receptor and the Delta/Serrate/Lag-2 (DSL) ligand of an adjacent cell initiates the Notch signaling pathway in the receptor-bearing cell [26]. Contrarily, cis-interactions between the receptors and ligands on a single cell lead to pathway suppression [27]. Upon the successive trans-interaction between Notch receptor and ligand, conformational change in the receptor occurs, allowing metalloprotease 10 (ADAM10) or 17 (ADAM17)/TACE (TNFα converting enzyme) mediated proteolytic cleavage at NECD site 2 (S2) [28]. This proteolytic cleavage produces the membrane-bound Notch extracellular truncation (NEXT) protein, which is further subjected to a second proteolytic cleavage at the NTM site 3 (S3) by γ-secretase [29,30]. γ-secretase consists of five subunits: presenilin 1, presenilin 2, nicastrin, presenilin enhancer 2 (Pen-2), and anterior pharynx-defective 1 (Aph1) [31–33]. Presenilin, an aspartyl protease, forms the catalytic subunit of the γ-secretase complex [34,35]. Nicastrin is required to maintain the stability of presenilin and helps regulate the intracellular trafficking of the complex [31,36,37]. Aph1 is required to support the proteolytic activities of the complex, and Pen2 is responsible for stabilizing the complex after proteolysis [38,39]. γ-secretase cleaves the Notch receptor in the plasma membrane or in endosomal compartments of the cell and releases the NICD into the cytoplasm. It has been reported that NICD produced from cleavage in the plasma membrane is more stable than that produced in the endosomal compartments [40,41]. The NICD translocates from the cytoplasm to the nucleus, where it binds to and activates the transcription factor CSL (also termed CBF1 or RBP-Jk), through which it transcribes Notch target genes.

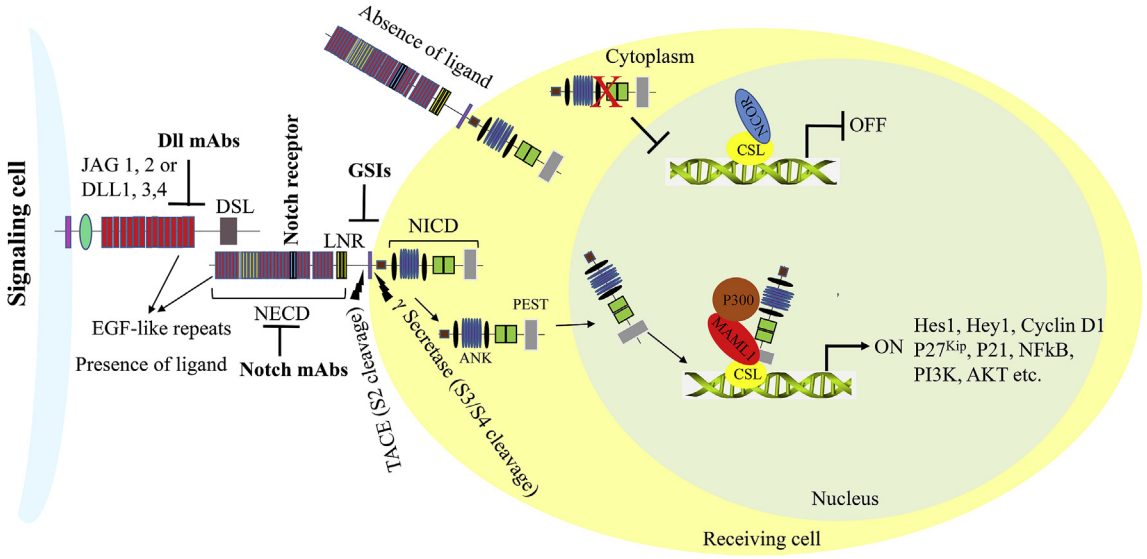

Fig. 3. Schematic representation of Notch receptor activation.
Notch receptors are activated upon binding to Serrate- and Delta-like ligands present on the cell membranes of adjacent cells. Following successful activation, Notch receptors undergo a series of proteolytic cleavages at site 2 (S2), mediated by metalloprotease 10 (ADAM10) and TACE (TNFα converting enzyme). Additional proteolytic cleavages at the transmembrane domain (NTD) are carried out by a multi-subunit complex, γ-secretase, at site 3 (S3). The Notch intracellular domains (NICDs) are then released into the cytoplasm. The NICDs further translocate into the nucleus, where they displace histone deacetylase and co-repressors in CSL repressor complexes and recruit MAML1 and histone acetyltransferase p300 to form active transcriptional complexes, which regulate the transcriptional activity of Notch target genes.

CSL represses the transcriptional activity of its target genes by binding to the DNA as part of a larger co-repressor complex consisting of HDACs, N-CoR, CSL interacting repressor (CIR) and SMRT/MINT/SPEN [42]. NICD interacts with CSL through its RAM domain and replaces the co-repressors in the CSL complex [42,43]. The interaction between the ANK domain of the NICD with CSL facilitates the recruitment of the co-activator Mastermind-like 1 (MAML1) [44]. This ternary complex then recruits other co-activators, such as p300 [45] and PCAF/GCN5 [46], through the C-terminal region of MAML1. These co-activators, in turn, convert CSL from a transcriptional repressor to a transcriptional activator. The NICD-CSL-MAML1-P300 complex mediates the transcription of Hes 1 (hairy/enhancer-of-split), Hey 1 (Hes-related with YRPW motif), Cyclin D1, p21, p27cip1/waf1, cMyc, Survivin, Slug, pre-Ta (pre-T cell receptor alpha chain), GATA3 and Nanog. The Notch signaling pathway also activates the nuclear factor-kappa B (NFκB) pathway [47] (Fig. 3).

4.2. Non-canonical pathway

Notch signaling can also be activated through non-canonical, ligand-independent pathways. Three types of non-canonical Notch pathways have been characterized: CSL-independent (Type I); S3 cleavage-independent (Type II); and Notch cleavage- and NICD release-independent (Type III) [48]. The functions of these non-canonical pathways have been identified predominantly in undifferentiated cell populations, such as stem/progenitor and embryonic/primordial cells, and they have been found to contribute to the maturation of both $CD4^+$ and $CD8^+$ single-positive thymocytes [49].

5. Role of the notch signaling pathway in breast cancer

Cellular functions are precisely monitored and tightly controlled in normal cells but not in cancerous cells. The dysregulation of developmental pathways has been correlated with several diseases, including cancer [50,51]. It has been established that organ development and tumorigenesis share similar mechanisms and that the Notch signaling pathway is crucial for embryonic development [52]. Studies suggest that developmental pathways such as Wnt, Hedgehog, and Notch were engaged in tumor cell development, progression, and survival [47,53,54]. Notch signaling plays an import role in the progression of several cancers, including BC [47,51,55]. The expression of the Notch receptors and their ligands was found to be highly elevated in BC tissues and correlated with poor survival of human BC patients [55–57]. In 1987, it was discovered that the Notch 4 locus is a common integration site for mouse mammary tumor virus (MMTV). This integration results in the constitutive ligand-independent activation of Notch 4, leading to the release of the NICD and thus increased activation of its target genes. These events facilitate the development of mammary adenocarcinoma [58]. Notch 1 was also found to be involved in the development of murine mammary tumors. Upon MMTV insertion, Notch 1 is truncated and can act as an oncogene [59]. Notch 1 is highly expressed in poorly differentiated breast tumors and it is associated with poor overall survival [55]. Interestingly, elevated levels of Notch 2 have been correlated with high rates of disease-free survival [60]. These observations suggest antagonistic functions of Notch 1 and Notch 2. The tumor-suppressing function of Notch 3 has also been suggested, as it upregulates Cyclin D1 in human BC cells and causes the accumulation of p27Kip, which leads to cell cycle arrest at the G0/G1 phase [61]. In contrast, triple-negative BC (TNBC) cells ectopically expressing Notch 4 showed increased proliferation and invasiveness, whereas inhibition/knockdown of Notch4 decreased the cell proliferation, invasion, tumor volume and tumorigenicity. Several studies have established that Notch signaling exhibits its oncogenic properties through its interactions with other signaling pathways, such as Ras, TGFβ, and Wnt in the mammary gland tumorigenesis [47]. Weijzen et al. demonstrated a significant correlation between the expression of Notch1 and H-Ras (a known oncogene) in human primary breast ductal carcinoma cases [62,63]. Notch signaling also regulates cellular processes, including apoptosis [51,64], angiogenesis [65,66], and the epithelial-to-mesenchymal transition (EMT) [67]. Notch prevents apoptosis in breast epithelial cells by inducing Akt signaling through the secretion of an autocrine signaling protein or the downregulation of PTEN expression [51,64,68].

Cancer stem-like cells/cancer-initiating cells (CSC/CIC) also play an important role in the initiation and metastasis of BC [69,70]. It is well known that Notch1 influences the self-renewal of breast CSCs/CICs by increasing ErbB2 transcription [71,72]. A comparison of activated Notch receptors in breast CSCs/CICs versus luminally differentiated $CD24^+$ cells indicated that Notch4 is highly activated in breast CSC-enriched cells [73]. These suggest that Notch pathway has a major participation and multiple roles during breast tumor progression.

6. Role of notch receptor ligands in breast cancer

Expression of the Serrate-like ligand Jagged 1 in cancer cells promotes angiogenesis in neighboring endothelial cells, and elevated levels of Jagged 1 have been associated with poor overall survival in human BCs [55]. Jagged 1-mediated Notch 1 activation inhibits E-cadherin expression through the induction of slug, thus promoting EMT in human breast epithelial cells [67]. Aberrant expression of Jagged 1 also induces bone metastasis of BC cells [74]. Dll1 is significantly up-regulated in ER^{+ve} luminal breast neoplasms, and its expression has been associated with poor prognosis of the same subtype. Intriguingly, Dll1 expression has shown no such effect in other BC subtypes [75]. Joana Sales-Dias et al. demonstrated the oncogenic properties of Dll1 in hormone positive BC cells [76]. Specifically, RNA interference-mediated downregulation of Dll1 in ER^{+ve} MCF7 BC cells resulted in reduced cell proliferation, migration, and colony formation. Kontomanolis et al. 2014 observed that the expression of Dll4 is highly correlated with metastasis in BCs. The authors investigated Dll4 levels in the plasma and neoplastic tissues of BC patients and found that patients with highly metastatic BC exhibited elevated levels of Dll4 in both [56]. This observation suggests that Dll4 plays a pivotal role in BC metastasis. Altogether, this mounting evidence clearly demonstrates that activation of the Notch pathway plays a key role in BC and is therefore a promising potential therapeutic target.

7. Notch signaling as a therapeutic target for cancer

7.1. γ-Secretase inhibitors

The aberrant activation of Notch signaling is highly correlated with carcinogenesis. The comprehensive study of the Notch pathway and its crosstalk with other oncogenic signaling pathways has provided enough evidence to identify potential therapeutic targets and to design effective strategies for the treatment of various cancers. The binding of ligands to NECDs triggers transmembrane cleavage of Notch receptors, which allows the release of NICDs into the cytoplasm. This proteolytic cleavage is carried out by γ-secretase. γ-secretase, a large, multi-subunit integral membrane protein complex, is important for the activation of Notch receptors and the transcriptional regulation of its target genes [15,29,30]. Thus, blocking transmembrane proteolytic cleavage using γ-secretase inhibitors (GSIs) could be a promising therapeutic approach (Table 1). GSIs prevent the generation of NICDs and thus inhibit Notch activity and its downstream events [77]. Most synthetic γ-secretase inhibitors have been developed to competitively inhibit presenilins. Z-Ile-Leu-CHO, popularly known as GSI-I, is a dipeptide that showed anticancer properties in Ras-transformed fibroblasts [61]. GSI-I was found to promote cell cycle arrest at the G2/M phase and to suppress BC cell survival, which further triggered apoptosis [78]. Recent reports suggest that GSI-I decreases cell proliferation by reducing the expression of Ki67 and glucose transporter 1 (Glut1), as well as by inhibiting the Notch and mTOR/Akt pathways [79]. Interestingly, the effects of

Table 1
List of Notch inhibitors.

Inhibitor name	Cancer type	Molecular target	Functions	Clinical Studies/Significance	Refs.
γ-secretase inhibitors (GSIs)					
Z-Ile-Leu-CHO	BC	Notch1, Bcl2, Bax and Bcl-XL	Arrest cells at G2/M phase leading to apoptosis	NA	[62]
LY411,575	BC	Notch1	Increase number of cells in G_2/M and G0 phase	Phase I clinical trials for Alzheimer disease	[82]
LY450139	BC	Notch1	Reduction of NICD and HES1	Phase I clinical trials for Alzheimer disease	[83]
MK-0752	BC and Solid tumors	γ-secretase	Reduce BCSCs	Phase Ib	[84]
RO4929097	BC	γ-secretase	Decreases NICD, HES1 expression	Phase I	[85]
PF-03084014	BC and T-ALL	γ-secretase	Reduction of NICD, HES1 and cMyc	Phase I	[86]
Monoclonal antibodies					
Anti-Dll4 mAbs (OMP-21M18)	Solid tumors	Dll4	Inhibits growth, anti-angiogenic	Phase II	[89,90]
Notch mAbs	T-cell leukemia cells	Notch 1	Reduction of NICD and HES1	NA	[93,94]
Natural Compounds					
Sulforaphane	BC and PC	Notch1	Increases chemo-sensitivity	NA	[97,98]
Genistein	BC	Notch1, NFκB and Capase3	Induces apoptosis	NA	[99,100]
Curcumin	BC and PaC	Notch1, NFκB	Induces apoptosis	NA	[101]
Quercetin	BC and PaC	Notch1	Arrest cell cycle at G0/G1 phase and induces apoptosis	NA	[102,103]

BC, Breast cancer; PC, Prostrate cancer; PaC, pancreatic cancer; T-ALL, T-cell acute lymphoblastic leukemia.

GSI-I are greater in $HER2^{+ve}$ cell lines than in $HER2^{-ve}$ BC cell lines [78,80,81]. Because trastuzumab, an inhibitor of HER2, can activate the Notch pathway, it would be interesting to investigate the combinatorial effects of trastuzumab and GSI-I in $HER2^{+ve}$ BC patients. LY411575 is a GSI that binds to presenilin 1 (PS1), induces apoptosis in $HER2^{+ve}$ BC cells, and re-sensitizes resistant $HER2^{+ve}$ cells to herceptin [82]. Several GSIs, including LY450139, MK-0752, PF-03084014, and RO4929097, have been or are currently being evaluated in phase I clinical trials [83–86]. RO4929097 has high selectivity and efficacy; however, for unknown reasons, it induces a “less transformed” and slower-growing tumor phenotype, rather than inhibiting cell proliferation or inducing apoptosis [85,86]. Despite progress in the field, poor pharmacokinetics and off-target effects present major drawbacks to the widespread use of these peptides in the clinic.

7.2. Monoclonal antibodies

Although GSIs have demonstrated strong potential in clinical trials, they fail to distinguish Notch paralogs. They inhibit all Notch receptors, which could be a disadvantage because some receptors may play tumor-suppressing roles that should not be inhibited. Furthermore, γ-secretase affects additional targets beyond the Notch pathways. For instance, γ-secretase cleaves β-amyloid precursor protein (APP), resulting in the accumulation of β-amyloid (Aβ) peptides that form plaques in the brain. GSIs might inhibit several such signaling pathways indiscriminately [87]. Indeed, the administration of GSIs has been found to cause intestinal toxicity in several other cancer types [88]. It will be important to discover new drugs with high specificity and affinity that can efficiently discriminate Notch receptor paralogs. In addition to GSIs, researchers have recently proposed a new therapeutic strategy to inhibit Notch signaling using monoclonal antibodies (mAbs) highly specific for Notch receptors and ligands (Table 1). Anti-Dll4 mAbs have been demonstrated to dysregulate tumor angiogenesis and growth by inhibiting the Notch signaling pathway in endothelial cells [89]. The humanized anti-Dll4 mAb (OMP-21M18) inhibits Notch signaling by blocking the interactions of Dll4 with Notch 1 and Notch 4 and was evaluated in clinical trials in patients with solid tumors. OMP-21M18 also showed anti-tumorigenic activity in patient-derived xenografts [90]. In contrast, some mAbs induce proteolytic cleavage of Notch 3 by binding to overlapping epitopes and mimicking ligand-induced Notch signaling activation [91]. mAbs against specific Notch receptors have also been developed and are under investigation [92]. Notch receptor-specific antibodies bind to the NECD and prevent ADAM10-mediated proteolytic cleavage [91,93,94]. These mAbs (OMP-59R5) have also shown promising anti-tumorigenic activity and are being tested in clinical trials [91]. Nicastrin mAbs were found to be efficient in the inhibition of γ-secretase and had anti-CSC and therapeutic activity in BC. However, these mAbs are also not specific to an individual Notch receptor [95].

7.3. Natural compounds

Natural compounds have gradually been gaining attention due to their anticancer activity. Consumption of citrus fruits, soybeans, and green cruciferous vegetables has been associated with reduced risk of cancer [96,97]. Natural compounds have shown promising results as chemopreventive agents in various cancer types, and their pleiotropic effects against cancer are under investigation. Several natural compounds, such as flavonoids and polyphenols, have demonstrated anticancer properties by inducing apoptosis and reducing the proliferation of various cancer types. Recent studies suggest that a few flavonoids also target the Notch signaling pathway (Table 1). The natural compound sulforaphane, derived from cruciferous vegetables, inhibits BC stem cell growth by down-regulating the Wnt/β-catenin self-renewal pathway *in vitro* and *in vivo*. Sulforaphane also inhibits the Notch 1 receptor. Moreover, it has been found to increase the sensitivity of

pancreatic cancer cells to chemotherapeutic agents such as gemcitabine, cisplatin, doxorubicin, and 5-fluorouracil [98,99]. The isoflavonoid genistein, derived from soy products, has exhibited anti-tumorigenic activity in pancreatic cancer and BC [99]. It was determined that genistein induced apoptosis in both ER^{+ve} and ER^{-ve} BC cells through caspase3 activation. In MDA-MB231 cells, genistein induced apoptosis by inhibiting NFκB via the Notch 1 receptor. Genistein-treated MDA-MB231 cells accumulated at the G2/M phase in a dose-dependent manner [100]. The well-known natural compound curcumin, derived from the roots of the Zingiberaceae family plant (i.e., Curcuma long), is a constituent of turmeric and is widely used as a flavoring agent in food. Curcumin inactivates NFκB by down-regulating Notch 1, inducing apoptosis in pancreatic cancer cells [101]. Quercetin is a polyphenol and flavonoid widely distributed in red grapes, apples, raspberries, citrus fruits, and green leafy vegetables. Quercetin decreased the expression of Notch 1 in a leukemia cell line and targeted pancreatic CSCs. Quercetin arrests the cell cycle at the G0/G1 phase and induces apoptosis in BC cells [102,103].

7.4. Notch receptors in tumor immune response

Although several studies suggested the tumorigenic properties of Notch receptors, there are few reports which also demonstrated the role of these receptors in the anti-tumor immune response. It has been reported that Notch receptors favor the differentiation of T-cell lineage over B cell development from the common lymphoid progenitor cells in the bone marrow [104,105]. CD4 T-helper 1 (TH1) cells and CD8 cytotoxic T-lymphocytes (CTL) play an important role in mediating anti-tumor immune response and Notch is found to be required for the activation and effector function of these cells [106]. Conditional activation of Notch 2 in CD8 T-cells induced an anti-tumor immune response and reduced the tumor burden in mice [107,108]. This suggests that, Notch signaling pathway is crucial for the activation and effector function of T-cells.

Tumor cells adopt several defensive mechanisms such as producing immunosuppressive cytokines, expressing inhibitory ligands and recruiting immunosuppressive myeloid and lymphoid cells into the microenvironment to evade the anti-tumor immune response [109]. To overcome this, researchers either isolated tumor antigen-specific T-cells from the tumor site or engineered using chimeric antigen receptors (CARs) specific for tumor antigens [110,111]. Recently, synthetic Notch receptors (synNotch) have been engineered to improve the generation and enhance the specificity of CAR T-cells [112–114]. These studies emphasize the importance of understating the role of Notch signaling pathway in T-cell-mediated anti-tumor immune response in order to design more effective T-cell-based immunotherapies.

8. Significance

Targeted therapies have emerged over the last decade as a new strategy for cancer treatment. The Notch signaling pathway, is one of the most commonly activated signaling pathways in cancer, plays an important role in cell differentiation, proliferation, angiogenesis, survival, and chemo-resistance, acting as an oncogene or tumor suppressor, depending on cellular context. Notch receptors bind to ligands present on adjacent cells, facilitate proteolytic cleavage by γ-secretase, and are released into the cytoplasm as NICDs, which translocate into the nucleus and regulate the transcriptional activity of target genes. The expression of several Notch receptors and ligands has been associated with the progression of several cancers, including BC, and correlated with poor prognosis.

Inhibition of the Notch signaling pathway using a number of promising approaches may provide a significant contribution to therapeutic strategies to treat BC. A few recent agents targeting Notch signaling are GSIs that inhibit all Notch receptors and have delivered promising results. Notch antibodies (currently under clinical trials) were developed to improve specificity and have exhibited successful tumor suppression. Some natural products have also been found to inhibit the Notch signaling pathway. In addition to GSIs, mAbs, and natural compounds, one of the most important Notch inhibition methods involves blocking peptides, which were also under clinical trials for the treatment of human malignancies. Interestingly, Notch receptors are found to be playing an important role in anti-tumor immune response [106]. Over expression of Notch receptors induce anti-tumor immune response by activating T cells and also reduce tumor burden in mice [107,108]. The present review suggests that Notch signaling pathway may be a promising therapeutic target for the treatment of BC.

9. Concluding remarks and future perspectives

Since recurrent BC is typically incurable, the propensity of BCs to recur following surgery, chemotherapy, and hormonal therapy is the most important determinant of clinical outcome. A role for Notch signaling in cancer progression and survival suggests that targeting this pathway alone or in combination with other pathways represents a promising therapeutic strategy. BC is a heterogeneous disease. Although 60% of BCs are hormone receptor-positive and receive anti-hormone therapy, they often develop resistance over time. On the other hand, hormone receptor-negative BCs are highly aggressive with minimal treatment options. There is an urgent need to understand the heterogeneity and complex molecular biology of BC in order to discover and develop the new therapeutic drugs to treat it. It is well known that the Notch signaling pathway plays an important role in BC survival, progression, cell growth, migration, invasion, and metastasis. Accumulating evidence and recent advancements in our understanding of Notch signaling indicate that it is a promising therapeutic target for the treatment of BC. To this end, it is important to understand the structure, function, and regulation of the Notch pathway, as well as its complex crosstalk with other signaling pathways.

Individual Notch family members may have opposing roles in cancer, depending on the cellular context and tumor type. For instance, highly elevated levels of Notch 1 and Notch 4 have been observed in BCs, and both have been categorized as oncogenes in several cancers. Surprisingly, Notch 3 was found to be a tumor suppressor. In addition to the Notch receptors, Notch receptor ligands Jagged 1 and Dll4 have been significantly associated with tumor angiogenesis. Following the successful interaction between a Notch receptor and a ligand on an adjacent cell, a series of proteolytic cleavages by TACE and γ-secretase are important for Notch pathway activation. Therefore, it would be wise to design GSIs as therapeutic drugs for the treatment of cancer. GSIs are novel compounds that can inhibit an important component of the Notch signaling pathway. The anticancer properties of GSIs in several cancer types are quite promising. However, GSIs fail to efficiently discriminate between Notch receptor, which is a major drawback. To address this specificity issue, mAbs have been developed to target specific Notch receptors or ligands and have been tested for their anti-tumorigenic effects in various cancer types. Anti-Notch1 and anti-Dll4 mAbs have strongly proven their efficiency and are under clinical trials. However, the Notch pathway interacts with several other oncogenic pathways, including PI3K/Akt, NFκB, and STAT3. Moreover, Notch receptors can have oncogenic or tumor-suppressing properties in various cancer types. Surprisingly, Notch receptors are shown to be effective in differentiation, activation of T cells in order to anti-tumor immune response. Synthetic Notch receptors were used to enhance the specificity of CAR T cells. This suggests that it will be impossible to achieve satisfactory therapeutic endpoints using Notch targeted monotherapy alone. Combinatorial treatments that include Notch inhibitors/mAbs in addition to traditional individual medicines may produce synergistically beneficial results in the clinical setting. Natural compounds, such as sulforaphane, genistein, curcumin, and quercetin, have also gained attention due to their anti-tumorigenic properties and

bioavailability and have shown promising results in several cancer types, including BC. Natural compounds showed anti-tumorigenic properties through modulating several oncogenic pathways including Notch signaling pathway, whereas Notch inhibitors will be able to inhibit single pathway. The bio-availability and stereospecificity of natural compounds is high comparing with the synthetic drugs. Natural compounds tend to show lesser side-effects than the synthetic drugs, which is an advantage. However, we need to study the possible roles of natural compounds thoroughly. Cumulatively, the past and ongoing research suggests that Notch signaling pathway may be a promising therapeutic target for the treatment of BC. However, it is important to consider the following aspects to successfully design a therapeutic Notch-targeting drug for the treatment of cancer: (i) specificity, (ii) affinity for a particular receptor or ligand, (iii) minimal efficacy:toxicity ratio, (iv) pharmacokinetics, (v) bioavailability, and (vi) inhibition of other oncogenic signaling pathways. Overall, here we summarize the current knowledge about the impact of the Notch signaling pathway in BC progression and the therapeutic role of Notch's inhibition.

Conflicts of interest

No conflict of interest exists for among the authors.

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