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Review Article

Furin, ADAM, and γ-secretase: Core regulatory targets in the Notch pathway and the therapeutic potential for breast cancer

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ABSTRACT

The activation of the Notch pathway promotes the occurrence and progression of breast cancer. The Notch signal plays different roles in different molecular subtypes of breast cancer. In estrogen receptor-positive (ER+) breast cancer, the Notch pathway regulates the activity of estrogen receptors. In human epidermal growth factor receptor 2-positive (HER2+) breast cancer, crosstalk between Notch and HER2 enhances HER2 signal expression. In triple-negative breast cancer (TNBC), Notch pathway activation is closely linked to tumor invasion and drug resistance. This article offers a comprehensive review of the structural domains, biological functions, and key targets of Notch with a specific focus on the roles of Furin protease, ADAM metalloprotease, and γ-secretase in breast cancer and their potential as therapeutic targets. We discuss the functions and mutual regulatory mechanisms of these proteinases in the Notch pathway as well as other potential targets in the Notch pathway, such as the glycosylation process and key transcription factors. This article also introduces new approaches in the treatment of breast cancer, with a special focus on the molecular characteristics and treatment response differences of different subtypes. We propose that the core regulatory molecules of the Notch pathway may become key targets for development of personalized treatment, which may significantly improve treatment outcomes and prognosis for patients with breast cancer.

Introduction

The Notch pathway plays an important biological role in the proliferation, differentiation, and apoptosis of tumor cells. Dysregulation of the Notch pathway is closely related to the occurrence, progression, chemotherapy resistance, and patient survival rate of breast cancer [1-3]. According to molecular characteristics, breast cancer is classified into three molecular subtypes: ER+/PR+; HER2+; TNBC [4]. Abnormal activation of the Notch pathway can promote the proliferation and metastasis of hormone receptor-positive breast cancer cells. Simultaneously, the Notch pathway is involved in the expression and functional regulation of hormone receptors, synergistically affecting the development and treatment response of breast cancer. Abnormal activation of the Notch pathway can also promote the proliferation and invasion of HER2-positive breast cancer cells, interacting with the HER2 signaling pathway to collectively promote the dissemination of tumor lesions. Interestingly, the Notch pathway plays multiple roles in TNBC, including regulating breast cancer stem cells (BCSCs), cell proliferation and

Abbreviations: ER+, estrogen receptor-positive; HER2+, human epidermal growth factor receptor 2-positive; TNBC, triple-negative breast cancer; BCSCs, breast cancer stem cells; ERα+, estrogen receptor α-positive; EMT, epithelial-mesenchymal transition; NECD, Notch extracellular domain; NICD, Notch intracellular domain; EGF, epidermal growth factor; ADAM, a disintegrin and metalloproteinase; PSEN, presenilin; NCSTN, nicastrin; PEN-2, presenilin enhancer 2; APH-1, anterior pharynx defective 1; CSL, CBF1 in humans, suppressor of Hairless in Drosophila and LAG in C. elegans; MAML, mastermind-like; GSIs, γ-secretase inhibitors; POFUT1, protein O-fucosyltransferase 1; Tregs, CD4+ T cells.

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survival, epithelial-mesenchymal transition (EMT), tumor invasion, and metastasis. Abnormal activation of the Notch pathway promotes tumor cells' resistance to chemotherapy drugs, reducing patients' survival time. In-depth research on the key targets of the Notch pathway, the regulatory relevance of potential targets with different molecular subtypes of breast cancer, and the development of Notch key target inhibitors will provide new options for personalized treatment of breast cancer.

Structure Domain of the Notch Receptor and Activation of the Notch Pathway

The Notch receptor has four subtypes, which are Notch-1, Notch-2, Notch-3, and Notch-4. Each receptor contains an extracellular domain (NECD), a transmembrane domain, and an intracellular domain (NICD). NECD contains multiple epidermal growth factor-like repeat sequences (EGF) domains and *Lin12*-Notch repeat sequences domains with cysteine residues, NECD binds to its ligands and activates Notch signaling. The NECD of Notch-1 and Notch-2 consists of 36 EGF-like repeat sequences, while the NECD of Notch-3 and Notch-4 contains 34 and 29 EGF-like repeat sequences, respectively. Transmembrane domain is composed of a short extracellular region with a pair of cysteine residues, and its function is to participate in heterodimerization. NICD contains the RBP-Jk binding domain, seven ankyrin repeat sequences, two nuclear localization signals, a transactivation domain, and a PEST sequence with rich in proline, glutamic acid, serine, and threonine residues [5]. The Notch ligand is also a transmembrane protein on the cell surface, which includes a Delta/Serrate/LAG-2 domain and multiple EGF domains [6]. It directly interacts with the Notch receptor between cells. There are five Notch ligands, including Jagged-1/-2 and Delta-like-1/-3/-4 [7]. The Delta/Serrate/LAG-2 domain of these ligands binds to the 11th and 12th EGF domains of the NECD structure of the Notch receptors. The specific interactions in these regions determine the activation of the Notch pathway [8], as shown in Fig. 1.

Activation of the Notch pathway undergoes three enzymatic cleavage steps. The Notch receptor is synthesized as an inactive single peptide precursor in the endoplasmic reticulum, which undergoes its first cleavage (S1) by a Furin protease in the Golgi network. S1 Cleavage generates an extracellular subunit and a transmembrane cytoplasmic subunit. In the absence of the ligands, these two subunits non-covalently bind to each other and form a mature Notch receptor with a heterodimer, which is then transported to the cell surface. After the Notch receptors bind to their ligands, a conformational change occurs, and the Notch receptor protein is cleaved for the second time by the ADAM metalloprotease (ADAM10/ADAM17) at the S2 site. The S2 cleavage leads to the dissociation of the extracellular domain and the generation of the Notch extracellular truncation intermediate. The third cleavage (S3) is mediated by the presenilin-dependent γ-secretase complex, leading to the release of active NICD into the cytoplasm. The γ-secretase complex consists of presenilin (homologous proteins PSEN1 or PSEN2), nicastrin (NCSTN), presenilin enhancer 2 (PEN-2), and anterior pharynx defective 1 (APH-1). When NICD translocate into the nucleus, it binds with the ubiquitous transcription factor CSL (CBF1 in humans, suppressor of Hairless in Drosophila and LAG in C. elegans), and converts the repressor complex into a transcription activation complex, initiating the transcription of downstream target genes [9]. Therefore, Furin protease, metalloproteinases (ADAM10/ADAM17), and γ-secretase are three key enzymes that participate in the activation of the Notch pathway, which are depicted in Fig. 2.

Notch signaling suppresses the progression of breast cancer

The abnormal activation of the Notch enhances the resistance of ER+ breast cancer cells to endocrine therapy

The Notch pathway plays an important role in the pathogenesis of hormone receptor-positive breast cancer. In estrogen receptor α-positive (ERα+) cells, estradiol inhibits Notch-1, and affects the distribution of Notch-1 in the cells, as shown in Fig. 3A. It has been shown that Notch-1 can act as a signaling protein to control proliferation and survival of

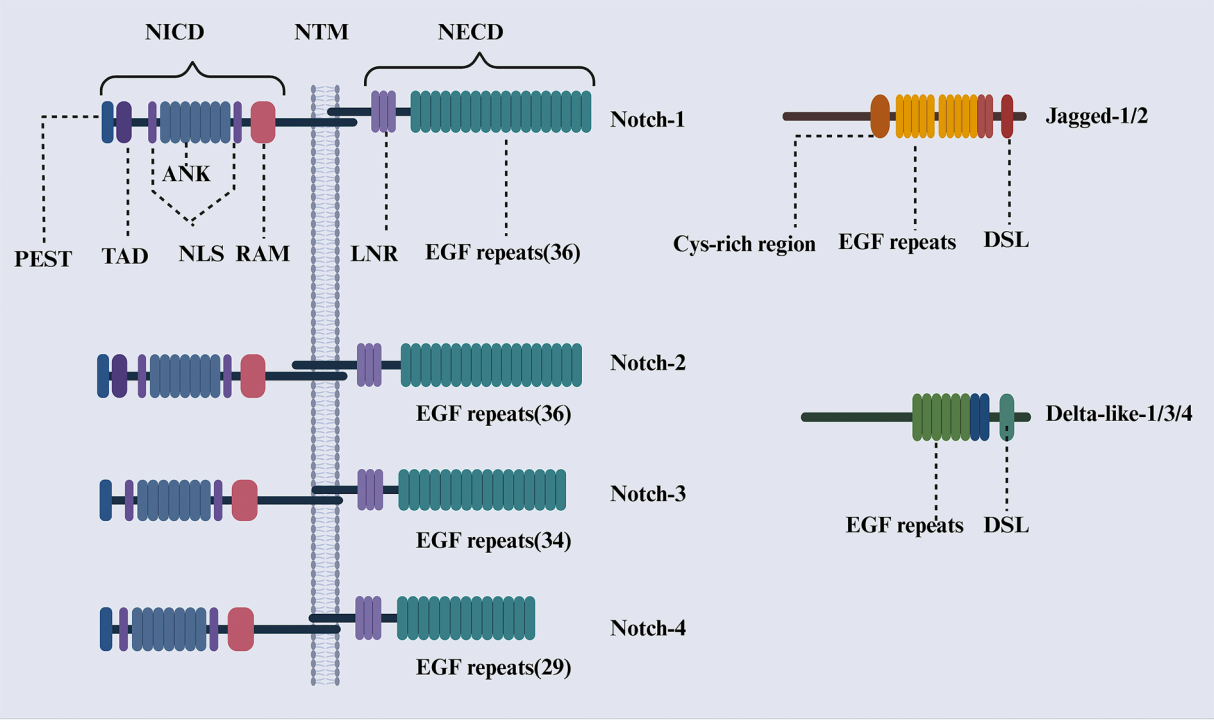

Fig. 1. Structure of Notch receptors and ligands. Notch receptors (Notch1-4) consist of extracellular, transmembrane, and intracellular domains. The extracellular domain of different Notch receptors contains varying numbers of EGF-like repeat sequences. Ligands (Delta-like -1, 3, 4 and Jagged- 1, 2) contain DSL (Delta/Serrate/LAG-2) and EGF-like repeat sequences in their extracellular domains, enabling them to interact with Notch receptors.

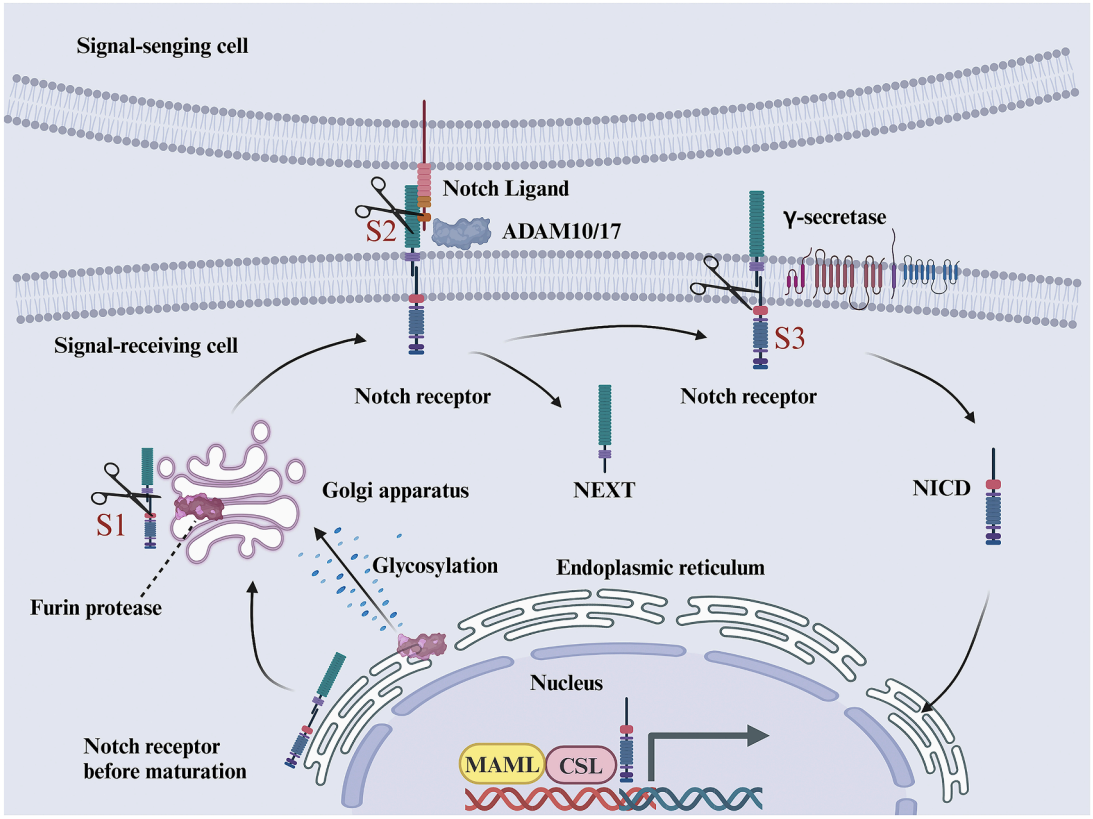

Fig. 2. Activation of the typical Notch pathway. Immature Notch receptors are transferred from the endoplasmic reticulum to the Golgi apparatus and are sequentially cleaved by three key Furin protease, ADAM proteases, and γ-secretase. Among them, Furin protease is synthesized as inactive precursors in the endoplasmic reticulum. They then undergo processes such as glycosylation modification and protein folding to achieve optimal conformation in the Golgi apparatus. When the Notch receptor binds to its ligand, ADAM10/17 cleaves at the S2 site, leading to the release of the NECD of the Notch receptor and the generation of the NEXT intermediate. Subsequently, Subsequently, γ-secretase cleaves at the S3 site, promoting the translocation of the NICD of Notch from the cell membrane to the nucleus, where it binds to CSL and recruit cofactors to initiate gene transcription and downstream gene expression.

ERα+ breast epithelial cells [10]. In ERα+ breast cancer cells, factors such as estrogen deprivation, loss of ERα+, or anti-estrogen therapy can reactivate Notch-1, induce Notch-4, and promote the proliferation, survival, and invasion of cancer cells [7]. Furthermore, the activation of the Notch pathway may lead to poor prognosis in ER+ breast cancer patients. For example, tumor-initiating cells can be enriched through Notch signaling and endocrine therapy, affecting the recurrence of estrogen receptor-positive breast cancer. Additionally, death-associated protein 6 is a newly discovered biomarker associated with ER+ breast cancer, which is negatively correlated with Notch in human ER+ breast tumor samples [11]. Another study found that prolactin synergizes with the canonical Wnt signal to drive the development of ER+ breast tumors by activating the Notch pathway [12].

The growth of ER+ breast cancer cells depends on estrogen stimulation, and at present endocrine therapy is still primary clinical approach for the treatment of the ER+ breast cancer [13]. The Notch pathway is an important target for the development and target of ER+ breast cancer. By interacting with ER signaling molecules, the Notch regulates the proliferation, differentiation and survival of ER+ breast cancer cells by affecting estrogen receptors. In addition, the Notch pathway regulates the growth and metastasis of ER+ breast cancer cells by activating its downstream molecules, such as PI3K/AKT and MAPK/ERK. Clinically, the patients with ER+ breast cancer tend to develop resistance to endocrine therapy, which is believed to be related to the abnormal activation of the Notch pathway [14]. The activation of the Notch pathway enhances the resistance of ER+ breast cancer cells to endocrine therapy, reducing the effectiveness of treatment. Therefore, the inhibition of the Notch pathway may overcome endocrine therapy resistance. The combination of Notch pathway inhibitors with endocrine therapy may synergistically enhance efficacy of ER+ breast cancer. In addition, the combination of Notch inhibitors with chemotherapy targeted therapy, or immunotherapy may also have synergistical improvement of clinical outcomes. It has to emphasize that although it has shown that the Notch pathway is a potential target for ER+ breast cancer, it is still in the research stage and has not been widely used in clinical practice.

The crosstalk between Notch pathway and HER2

Clinically, approximately 15-20% of breast cancer patients have HER2 overexpression, which is identified as a highly aggressive subtype of breast cancer [15]. HER2+ breast cancer often involves the amplification of the ERBB-2 proto-oncogene on chromosome 17 [16], leading to the production of a large amount of HER2 receptors in tumor cells, promoting tumor growth and metastasis. It has been shown that the Notch-1-PTEN-ERK-1/2 signaling pathway can promote the proliferation and survival of HER2+ breast cancer cells [17]. The presence of Notch binding sequences in the HER2 promoter indicates there is an interaction between Notch and HER2 pathway [18]. When the Notch pathway is activated, the NICD enters the nucleus and induces the transcription of target genes, such as HER2, leading to an increase in HER2 expression, which further activates the PI3K/Akt pathway and promotes the self-renewal of BCSCs [19], as shown in Fig. 3B. HER2/neu is a tumor-associated antigen that is overexpressed in 10-40% of breast cancer and other cancer cells [20]. Interestingly, the Notch signaling also plays a key role in the recurrence of breast cancer after HER2/neu inhibition. Studies have revealed that Notch activity is positively correlated with the recurrence rate of breast cancer patients, and the

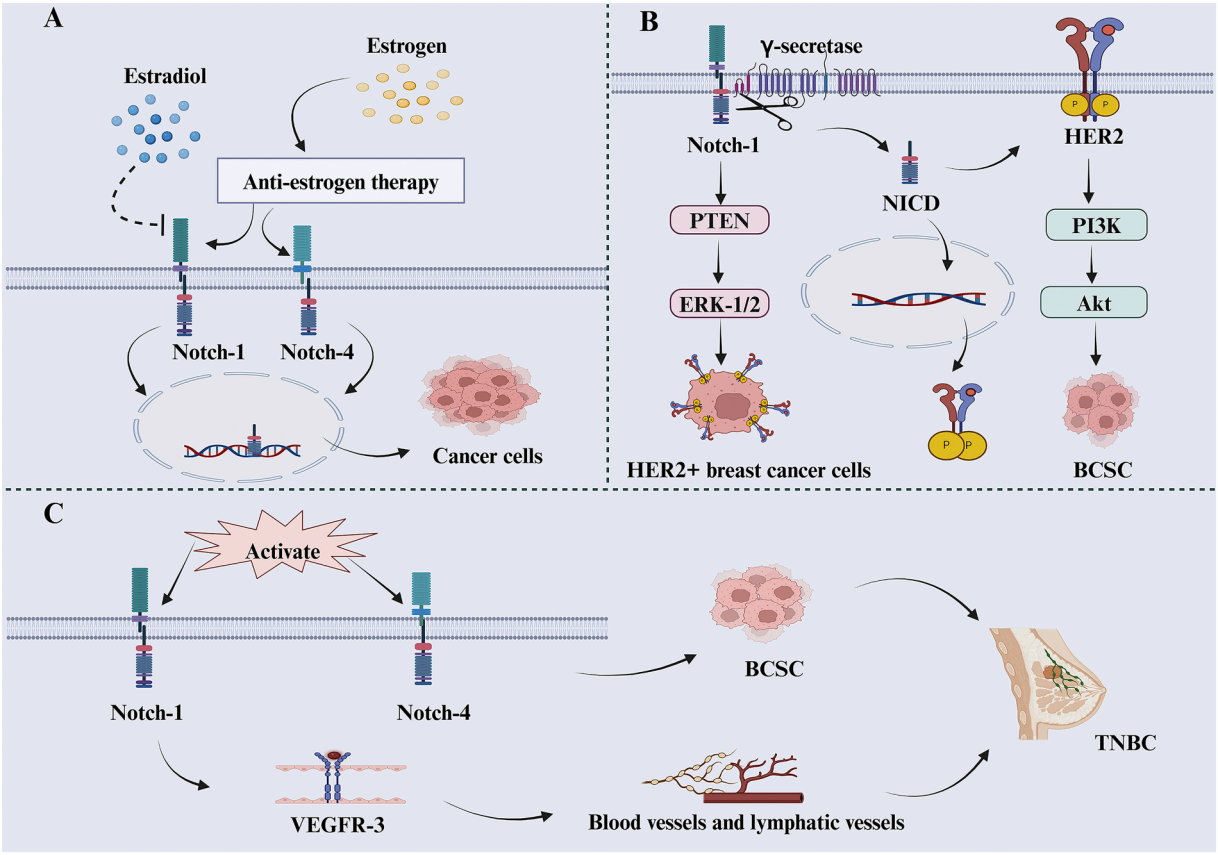

Fig. 3. Notch pathway's roles vary across breast cancer subtypes. A: In ERα+ cells, estradiol can inhibit Notch-1, thereby affecting the distribution of Notch-1 within the cells. In addition, factors such as anti-estrogen therapy can reactivate Notch-1, induce Notch-4, and promote the proliferation, survival, and invasion of cancer cells. B: The Notch-1-PTEN-ERK-1/2 signaling pathway can promote the occurrence of HER2+ breast cancer, and there is an interaction between the Notch and HER2 signaling pathways. C: Notch-1 and Notch-4 receptors can be overexpressed in vascular endothelial cells and TNBC.

abnormal activation of Notch promotes the recurrence of HER2/neu-driven breast tumors in genetically engineered mice [21]. The Notch pathway is involved in regulating the self-renewal ability of HER2+ BCSCs. Studies have shown that inhibiting Notch-1 can reduce the self-renewal ability of HER2+ BCSCs [22].

Interestingly, there is an interaction and cross-feedback between the Notch pathway and the HER2 pathway [23]. The overexpression of HER2 activates the Notch pathway, promoting the growth and survival of tumor cells. The activated Notch pathway in turn further activates the HER2 pathway, leading to resistance of tumor cells to HER2-targeted drugs. It has been shown that the interaction between the Notch pathway and the HER2 pathway can affects the sensitivity of HER2+ breast cancer cells to HER2-targeted drugs. Targeting this process may be a new therapeutic strategy to overcome tumor resistance and improve the efficacy of anti-tumor drugs. Therefore, it is tempted to develop novel therapeutic approaches or compounds that target Notch pathway or simultaneously inhibit the Notch and HER2 pathways. These therapeutic approaches are expected to provide more personalized and effective treatment options for HER2+ breast cancer patients.

The abnormal activation of the Notch pathway enhances the invasion and chemoresistance of TNBC

TNBC is a highly invasive breast cancer, accounting for 20% of all breast cancers [24]. The clinical metastasis and recurrence rate is high with a trend of younger onset. Abnormal activation of the Notch pathway plays a crucial role in TNBC. Research shows that Notch-1 and Notch-4 receptors are overexpressed in vascular endothelial cells and TNBC, while Notch-1 regulates the expression and activity of vascular endothelial growth factor receptor 3, and influences blood vessel development and lymphatic vessel formation, thereby accelerating the progression of TNBC [25]. In MDA-MB-231 cells, Notch-1 increases the expression of the major vault protein, which stimulates the AKT pathway, leading to the promotion of EMT and the induction of TNBC chemotherapy resistance in TNBC [26]. TNBC cells also have higher expression of Notch-4, which may increase the enrichment of BCSCs in TNBC cells [27], thereby affecting tumor recurrence and drug resistance, as shown in Fig. 3C. Silencing Notch-4 lowers the metastatic ability of TNBC cells, and enhances the tumorigenic ability of TNBC [28]. Jagged-1 is a ligand of the Notch pathway, which interacts with the Notch receptor to activate the pathway, thereby regulating cell fate, proliferation, and differentiation. It has been found that Jagged-1 increases the microenvironmental angiogenesis in TNBC by promoting exosome secretion and the activation of MALAT1-miR-140-5p-JAG-1/VEGFA pathway, thereby inducing drug resistance in TNBC [29].

Currently, there are no effective targeted drugs available for TNBC treatment. It has been shown that γ-secretase inhibitors (GSIs) can inhibit the proliferation of tumor cells and promote apoptosis by regulating the Notch pathway [30]. In addition, γ-secretase/Notch pathway may be involved in TNBC resistance, as inhibiting this pathway can reverse the resistance of TNBC, and enhance the efficacy of chemotherapy drugs [31]. In the future, the development of GSIs from natural sources in combination with other drugs may offer new ideas and strategies for the treatment of TNBC, and GSIs may be one of the most promising drugs for targeted drug screening and treatment of TNBC.

Regulation of breast cancer by Furin protease

Furin protease can promote the maturation of the Notch receptor

Furin is a type I transmembrane protein of the Bacillus subtilis protease-like convertase family that contains 794 amino acids and is present in all vertebrates [32]. The structure of the Furin protease includes a signal peptide, a pro-domain, a subtilisin-like catalytic domain, a middle P domain, a cysteine-rich region, a transmembrane helical domain, and a cytoplasmic domain [33] (Fig. 4). Among them, signal peptides can guide Furin protease into the endoplasmic reticulum. The pro-domain is the inhibitory region of Furin protease, which is hydrolyzed through self-catalytic cleavage. The subtilisin-like catalytic domain is the active region of Furin protease, responsible for cleaving precursor proteins. The middle P structural domain participates in substrate recognition and binding processes of Furin, ensuring accurate cleavage by Furin protease and regulating its function. Cysteine-rich region play a crucial role in the stability and functional regulation of Furin protease. The transmembrane helical domain enables Furin protease to embed in the cell membrane, and the cytoplasmic domain is associated with intracellular signal transduction regulation [34]. Furin protease is synthesized in the endoplasmic reticulum and transported to the plasma membrane via the endoplasmic reticulum-Golgi pathway [35]. Specifically, the Furin protease undergoes a series of transport and modification processes in the endoplasmic reticulum. The Furin protease is synthesized as an inactive precursor form in the endoplasmic reticulum, where it undergoes processes such as glycosylation modification and protein folding to obtain the correct structure in the Golgi apparatus. Then, the Furin protease is transported to the plasma membrane through the endoplasmic reticulum-Golgi apparatus. During this process, the Furin protease is packaged into transport vesicles and transported to the plasma membrane through the Golgi apparatus, which exits the complex along the intracellular membrane system. Furin protease preliminarily cleaves Notch receptors in the Golgi apparatus, causing them to mature. Mature Notch receptors are then transported to the plasma membrane through transport vesicles, bind to ligands, and activate the Notch signaling pathway [5,35].

Furin protease promotes the proliferation, invasion, and metastasis of breast cancer cells

Research has found that the interaction between Furin protease and Placenta-specific protein 1 (Plac1) promotes the invasion and metastasis of breast cancer cells. Plac1 forms a complex with Furin protease and interacts each other inside the cells. Additionally, Plac1 degrades Notch-1 and generates the intracellular domain of Notch-1, thereby inhibiting the activity of PTEN. These findings suggest that the functional interaction between Plac1 and Furin protease enhances the invasion and metastasis of breast cancer, and also reveal that the Furin protease/NICD/PTEN signaling pathway may become an important target for treating breast cancer [36] (Fig. 5A). The Furin protease can influence the biological behaviors of breast cancer cells by regulating the intracellular calcium ion concentration. Inhibiting Furin protease elevates the concentration of intracellular calcium ions, effectively suppressing the proliferation and invasive capabilities of breast cancer cells [37]. Interestingly, the Furin protease promotes the maturation of the insulin-like growth factor-1 receptor (IGF-1R) in breast cancer cells, thereby enhancing the proliferative capacity of the cells [38] (Fig. 5B). Lipoic acid (LA) is a natural compound that inhibits the maturation process of IGF-1R in breast cancer cells, and further inhibiting the development of breast cancer. Recent studies have shown that the expression of Furin protease is higher in TNBC cell lines. Specifically, Furin protease acts by cleaving and activating important protein precursors, including receptors IGF-1R and IR (insulin receptor) that are

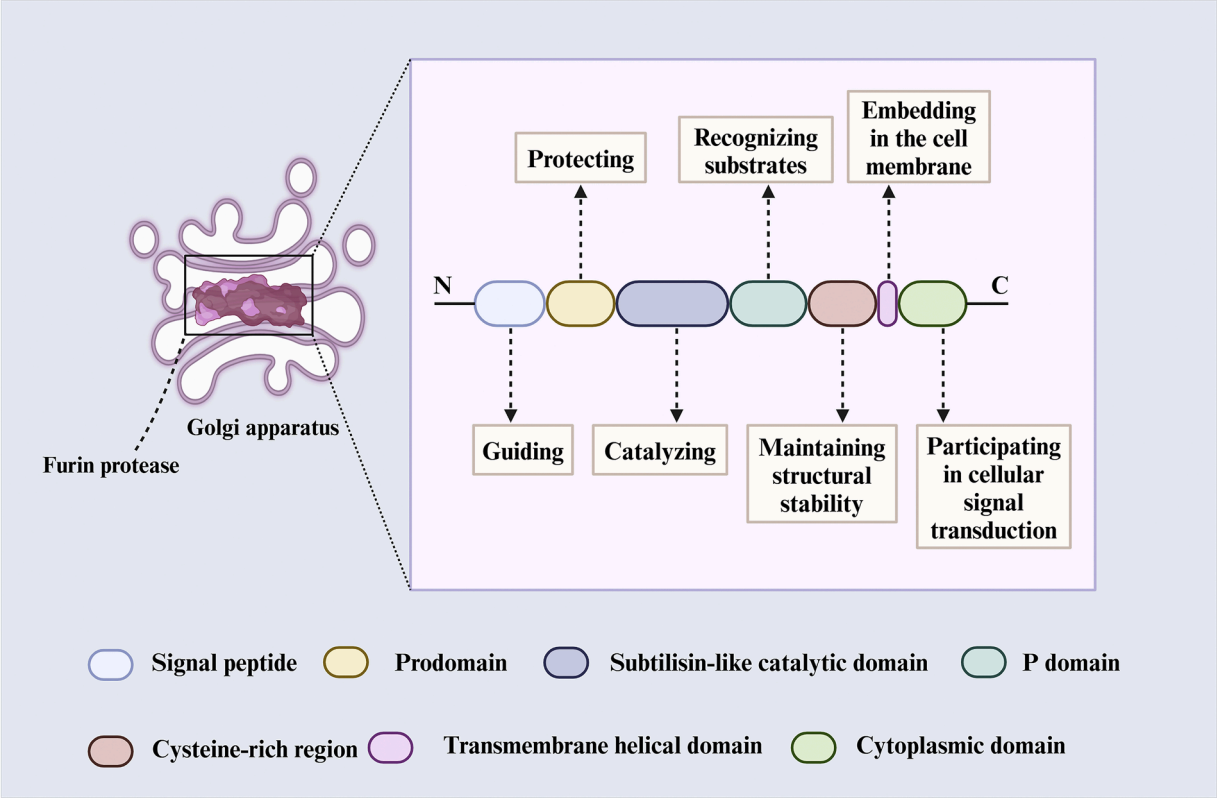

Fig. 4. The cellular location and structural domain of the Furin protease. Furin is primarily located in the trans-Golgi network within cells. The different structural domains of Furin protease are responsible for guiding, protecting, catalyzing, recognizing substrates, maintaining structural stability, embedding in the cell membrane, and participating in cellular signal transduction.

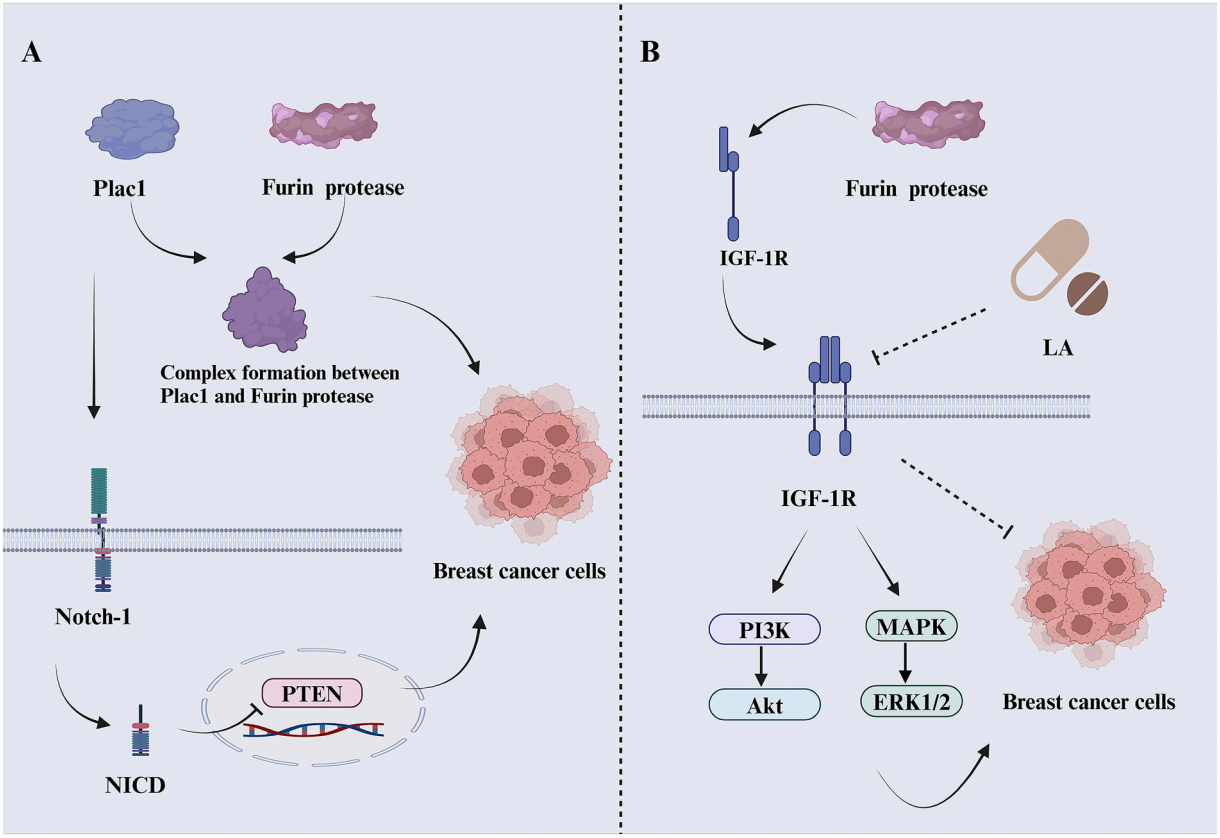

Fig. 5. A: Furin protease interacts with Plac1, promoting invasion and metastasis of breast cancer cells. The Furin protease/NICD/PTEN signaling pathway may become an important target for treating breast cancer. B: Furin protease promotes the maturation of IGF-1R, enhancing the proliferative ability of breast cancer cells. LA can inhibit the maturation process of IGF-1R in breast cancer cells, further inhibiting the development of breast cancer.

involved in the occurrence and development of TNBC. Inhibiting Furin protease affects the cleavage and activation of these receptors, thereby affecting the activation of downstream signaling pathways (such as PI3K/AKT and MAPK/ERK1/2 pathways), while these pathways are closely related to the proliferation, invasion, and metastasis of TNBC [39]. Therefore, the Furin protease plays an important regulatory role in the proliferation, invasion, and metastasis of breast cancer cells, and targeting Furin protease may become a potential strategy for breast cancer treatment.

The inhibition of Furin protease as a potential strategy for breast cancer treatment

Immunotherapy targeting Furin-related antigens as a novel therapeutic strategy has promising prospects in the treatment of breast cancer. Recent studies have shown that Furin protease in immune cells is associated with regulating immune responses and tumor growth, the absence of Furin protease in T cells can lead to regulatory CD4+ T cells (Tregs) dysfunction, thereby affecting the onset and progression of TNBC. The loss of Furin protease prevents the proper cleavage activation of transforming growth factor (TGF) β1 precursor, thereby affecting the differentiation and proliferation of Tregs [40]. Targeting Furin protease can inhibit Tregs' function, enhance the anti-tumor response of CD8+ T cells, and thus inhibit the growth and metastasis of TNBC. PEA II is an immune pro-apoptotic protein that induces apoptosis of HER2+ tumor cells [41]. After being cleaved by the Furin protease, PEA II undergoes translocation and enters the cell nucleus, thereby inducing cell death. This study reveals that Furin protease-mediated cleavage plays a crucial role in the pro-apoptotic activity of immunotoxins, providing an important theoretical basis for the development of novel therapeutic strategies for HER2-positive breast cancer.

It is worth mentioning that autologous cancer vaccine may also be a potential cancer therapy, which involves genetically modifying cancer cells in patients, and the injection of them into the patient's body to trigger an anti-tumor immune response. The study on one patient who received the injection of autologous cancer vaccines that knock out the Furin protease gene and enhance GM-CSF (Granulocyte-macrophage colony-stimulating factor) [42], has shown that autologous cancer vaccine can promote the immune system's response to tumors and improve the effectiveness of treatment. However, this therapy is still under the research stage, and clinical studies are needed to confirm its effectiveness and safety in the field of cancer treatment.

Regulation of breast cancer by ADAM metalloprotease

ADAM's abnormal activation increases the release of the Notch extracellular domain

It has been shown that the ADAM metalloprotease family, particularly ADAM10 and ADAM17, activates the Notch pathway by cleaving the Notch receptor to release the NECD. Typical ADAM protein domains include the N-terminal signal sequence, pro-domain, metalloproteinase domain, disintegrin domain, cysteine-rich region, EGF-like domain, transmembrane domain, and cytoplasmic domain [43] (Fig. 6). Among them, the N-terminal signal sequence is responsible for guiding the inactive ADAM metalloprotease precursor into the secretory pathway [44]. The pro-domain helps maintain the ADAM metalloprotease in an inactive state, and is cleaved by proprotein convertases (such as Furin protease) in the secretory pathway. The metalloproteinase domain binds to zinc ions through a sequence with three histidine residues and one glutamic acid residue, catalyzing the cleavage of peptide bonds [45]. The disintegrin and metalloproteinase domain are present in all

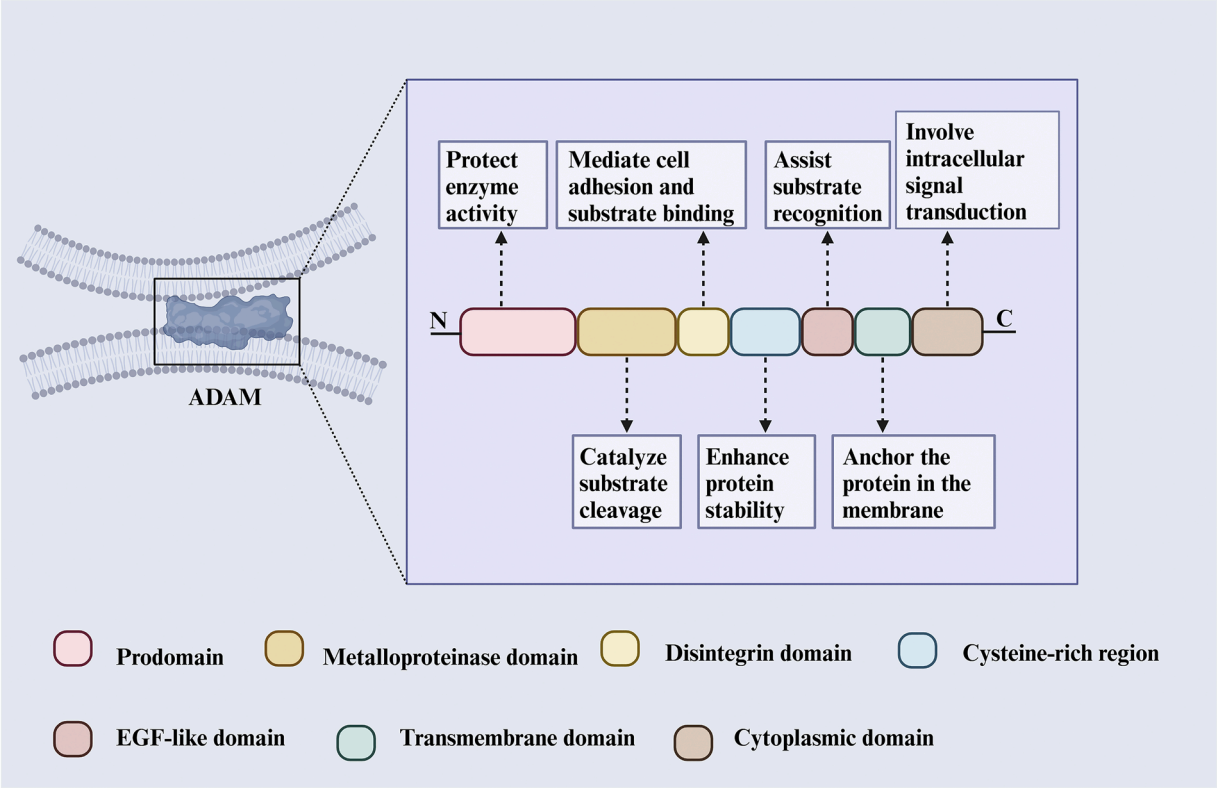

Fig. 6. The cellular location and structural domain of the ADAM. ADAM typically exists on the surface of cells or is secreted into the extracellular space. The N-terminal signal sequence directs localization, the pro-domain protects enzyme activity, the metalloproteinase domain catalyzes substrate cleavage, the disintegrin domain mediates cell adhesion and substrate binding, the cysteine-rich region enhances stability, the EGF-like domain aids substrate recognition, the transmembrane domain anchors the protein, and the cytoplasmic domain is involved in intracellular signaling.

members of the ADAM family and are involved in cell adhesion, migration, and signal transduction [46]. The region rich in cysteine plays a key role in mediating interactions between protein-protein and regulating the strength and timing of signal transduction in the Notch pathway. The EGF-like domain mediate cell signaling transduction by binding to and activating the EGF receptor. It is worth mentioning that ADAM10 and ADAM17 do not contain EGF-like domains [47]. The transmembrane domain is responsible for anchoring the ADAM protein to the cell membrane. The cytoplasmic domain contains signaling motifs, such as phosphorylation sites or regions rich in proline, which bind to the tyrosine kinase homology-3 domain. This binding interaction plays an important role in signal transduction and the interaction of the cell cytoskeleton [43]. ADAM10/17 mainly regulates the Notch pathway by cleaving the Notch receptor and Notch ligand. The Notch receptor is a transmembrane protein, when the Notch receptor binds to its ligand, ADAM10/17 will cleave the S2 site of the Notch receptor, leading to the release of the NECD, this cleavage process is known as "ectodomain shedding". The cleavage of NECD exposes the NICD of the Notch receptor, which further interacts with the γ-secretase complex to activate Notch signal transduction [48]. The main roles of ADAM10 and ADAM17 in the Notch pathway are to activate Notch signal transduction by cleaving the extracellular domain of the Notch receptor. Their activity and cleavage effects have a significant impact on the strength and timing of Notch signal transduction.

ADAM promotes the infiltration of breast cancer cell, regulates angiogenesis and tumor microenvironment

ADAM17 participates in the infiltration process of breast cancer cells by promoting cell migration, proliferation, and angiogenesis. ADAM17 cleaves and releases epidermal growth factor receptor (EGFR) ligands (such as TGF-α) and activate EGFR signaling which activates the PI3K-AKT signaling pathway and promotes tumor progression. Overexpression of ADAM17 increases phosphorylation of EGFR and AKT, enhancing the proliferation and invasive capabilities of breast cancer cells [49] (Fig. 7A). In addition, ADAM17 is also involved in the process of angiogenesis in breast cancer. It can release angiogenic factors, such as vascular endothelial growth factor (VEGF), thereby promoting the formation of new blood vessels to provide sufficient nutrients and oxygen for the tumor [50]. Interestingly, in breast cancer cells with HER2 overexpression, ADAM10 cleaves the HER2 receptor, releasing the extracellular domain of HER2, generating a fragment with sustained kinase activity (Fig. 7B). This process is clinically significant because serum levels of HER2 ECD in metastatic breast cancer patients are associated with a poor prognosis [51]. Other studies have shown that the expression of ADAM10 is closely related to the invasive ability of breast cancer cells. Blocking the expression or activity of ADAM10 through RNA interference or small molecule inhibitors can significantly reduce the invasive ability of breast cancer cells, suggesting that ADAM10 may be a key regulatory molecule for the invasion of breast cancer cells [52]. In addition, glycoprotein non-metastatic melanoma protein B/osteoactivin (GPNMB/OA) is a transmembrane protein, its primary function is to promote tumor growth, invasion, and metastasis. After being cleaved by ADAM10, GPNMB/OA releasing its soluble ectodomain into extracellular compartment, which stimulates the migration of endothelial cells and promotes angiogenesis in breast cancer tissues [53] (Fig. 7B).

New approaches for the treatment of breast cancer with ADMAs as the target molecule

In addition to metalloproteinases ADAM10/17 as target of breast cancer, several other ADAM metalloproteases in Notch pathway, such as

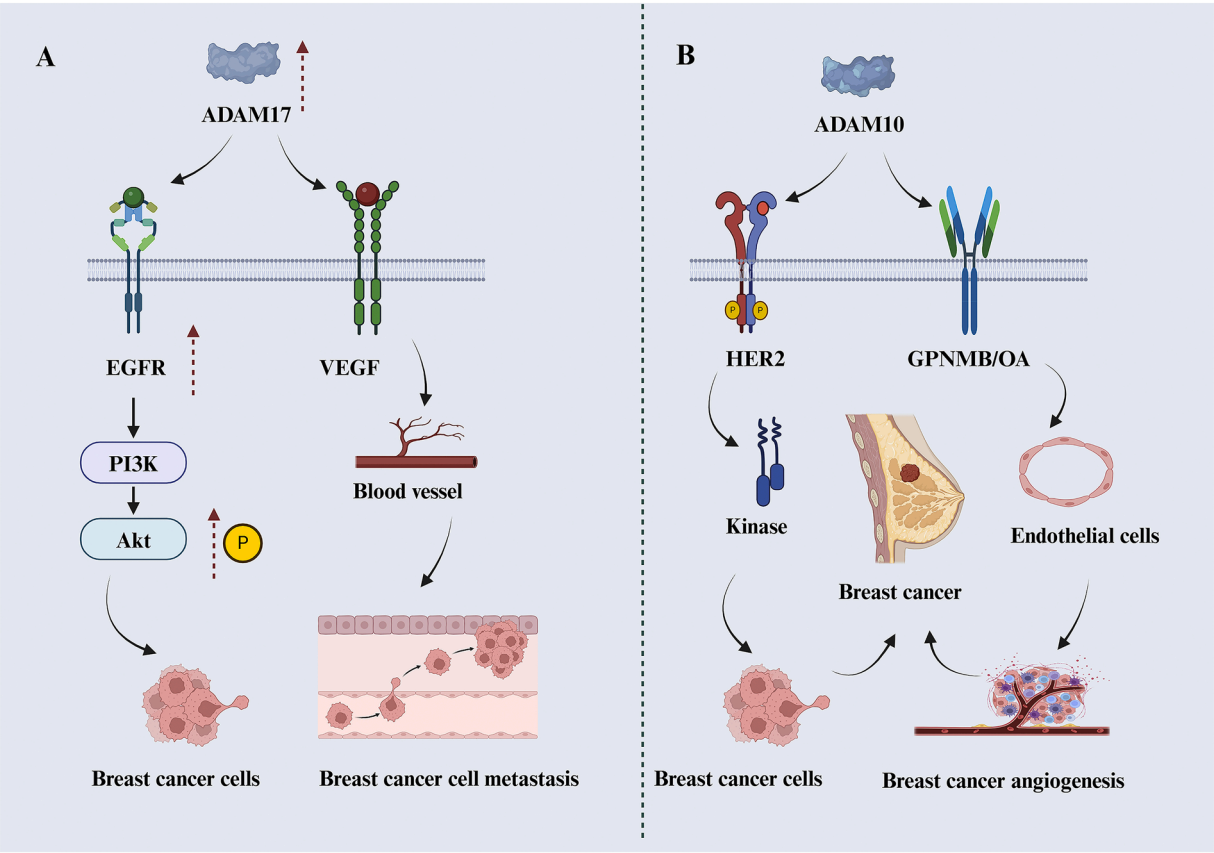

Fig. 7. A: ADAM17 cleaves and releases EGFR ligands, activating the PI3K-AKT signaling pathway, promoting cancer cell proliferation, invasion, and metastasis. ADAM17 also participates in the angiogenesis process of breast cancer, releasing VEGF to promote new blood vessel formation. B: ADAM10 cleaves HER2 receptors and GPNMB/OA, promoting breast cancer cell infiltration and angiogenesis.

ADAM8, ADMA9, ADMA23 and ADAM33, may also be considered as targets of breast cancer. ADAM8 has been shown to play an important role in breast cancer, especially in TNBC. ADAM8 can activate β1 integrin to make breast cancer cells to adhere to endothelial cells and enter the blood, increasing the risk of distant organ metastasis [54]. In addition, ADMA8 stimulates angiogenesis, regulates cell adhesion, and migration, thereby promoting breast cancer progression. Overexpression of ADAM8 increases invasiveness and metastatic potential of breast cancer. It has been shown that ADAM9 is associated with the invasiveness of TNBC. The expression of ADAM9 in patients' breast tissue or TNBC cells is negatively correlated with survival rates in TNBC patients. Inhibiting ADAM9 can significantly suppress the proliferation, migration, and invasion capabilities of TNBC cells by inactivating the AKT/NF-κB signaling pathway [55]. Another study found that high methylation of ADAM23 is associated with a poorer prognosis in breast cancer. Breast cancer patients with higher levels of ADAM23 methylation have shorter disease-free survival. Furthermore, the expression of ADAM23 is negatively correlated with EMT markers, suggesting that ADAM23 may inhibit breast cancer metastasis and invasion by regulating the EMT process [56]. It has been reported that ADAM33 is downregulated by DNA hypermethylation in TNBC, suggesting that this protease could serve as a biomarker for TNBC and other basal-like breast cancers [57]. Therefore, ADAM8, ADAM9, ADAM23, and ADAM33 may be potential targets for breast cancer, especially ADAM8 and ADAM9. These two proteins may serve as a potential target for developing small molecule inhibitor to treat TNBC.

Regulation of breast cancer by γ-secretase

The activation of Notch downstream target genes by γ-secretase

γ-secretase is a transmembrane protein complex with four key subunits: PSEN1 or PSEN2, NCSTN, PEN-2, APH-1 [58]. PSEN1 or PSEN2 is a core component of γ-secretase complex, containing multiple transmembrane domains. Their main function is to provide the active catalytic subunit, which hydrolyzes substrate proteins within the cell membrane [59]. NCSTN is an auxiliary subunit of the γ-secretase complex that can interact with PSENs. NCSTN mainly performs two functions: maintaining the structural stability of γ-secretase complex, and participating in substrate recognition and binding. The main function of PEN-2 is to promote the assembly and stability of γ-secretase complex, and participate in the correct folding and post-translational modification of presenilin [60]. APH-1 is another auxiliary subunit of γ-secretase complex. APH-1 interacts with PSENs to regulate the activity and stability of γ-secretase complex [61]. In the Notch pathway, the cleavage of the Notch receptor by γ-secretase depends on the cleavage of NECD by the ADAM proteinase. Specifically, the cleavage of NECD by ADAM10/17 generates two fragments: the N-terminal cleavage product and the C-terminal cleavage product. The N-terminal cleavage product is engulfed by ligand-expressing cells, while the remaining C-terminal fragment of the Notch receptor is cleaved by γ-secretase. This process promotes the translocation of the NICD from the cell membrane into the nucleus. In the nucleus, NICD binds to CSL transcription factors and recruits the nuclear transcriptional co-activator family of mastermind-like (MAML). This interaction induces the transformation of CSL transcription factors from transcriptional repression to activation, ultimately enhancing the expression of Notch (Hes, Hey) targeted genes

and its downstream genes [31].

γ-secretase promotes the proliferation of breast cancer stem cells and regulates the tumor microenvironment

NCSTN is a crucial subunit of the γ-secretase complex, playing a regulatory role in BCSCs and tumor growth. Studies have shown that the overexpression of NCSTN promotes EMT in breast cells, enhancing their invasive and migratory abilities. Moreover, the overexpression of NCSTN can increase the proportion of BCSCs, which helps maintain their undifferentiated state and increase the expression of pro-invasive genes and the invasion capabilities of tumor cells (Fig. 8). These effects are primarily regulated through the Notch-1 signaling pathway, partially mediated by the Notch-4 and Akt pathways [62]. In addition, γ-secretase cleaves the intracellular domain of CD44 protein (CD44-ICD), promoting the activation of stem cell factors (such as Nanog, Sox2, and Oct4), thereby maintaining the "stemness" of BCSCs [63] (Fig. 8). Research has found that γ-secretase-mediated CD44 intramembrane cleavage plays an important role in the migration of breast cancer cells. The interaction between CD44 and the extracellular matrix (ECM) promotes cell migration. Hyaluronic acid (HA) is a crucial component of the ECM, CD44 binds to HA and participates in the assembly of the HA-enriched extracellular matrix. Cleavage of CD44 is necessary for efficient cell migration in a matrix containing HA [64]. ERBB4 is a receptor tyrosine kinase, and γ-secretase can cleave ERBB4 to release its intracellular domain (ICD). ERBB4 ICD enters the nucleus and interacts with various transcriptional factors to regulate gene expression [65]. Moreover, ERBB4 ICD interacts with transcription factors STAT5, YAP, STAT5a, estrogen receptor beta, and HIF-1α (Fig. 8), to regulate the differentiation of mammary gland epithelium [66]. Therefore, γ-secretase cleavage of ERBB4 produces ICD, which plays an important regulatory role in the proliferation and differentiation of breast cancer cells. In breast cancer cells and the tumor microenvironment, γ-secretase has been found to be involved in the regulation of E-cadherin cleavage. E-cadherin is a cell adhesion protein that is crucial for maintaining cell adhesion and tissue structure stability. Studies have shown that the activity of γ-secretase is heightened in breast cancer cells, leading to the cleavage and degradation of E-cadherin. This process leads to the release of E-cadherin from the cell surface, disrupting cell adhesion, and facilitating the invasion and metastasis of tumor cells [67].

New strategy to inhibit γ-secretase and block the progression of breast cancer

The combination of GSIs with other drugs has great potential for the treatment of TNBC. It has been shown that combination therapy of γ-secretase inhibitor MK-0752 and docetaxel can reduce the accumulation of BCSCs and enhance the efficacy of docetaxel on TNBC [68]. A clinical trial shows that the combination therapy of RO-4929097 and neoadjuvant paclitaxel and carboplatin has beneficial therapeutic effects in TNBC patients [69]. Research shows that the combination of PF-03084014 and docetaxel can produce a synergistic effect in a TNBC model, enhancing the treatment efficacy for TNBC and reducing the number of tumor stem cells [70]. In addition, the combination therapy of RO4929097 and Exemestane has entered clinical trials for the treatment of ER+ metastatic breast cancer. This treatment has shown promise in controlling the progression of ER+ metastatic breast cancer to some extent [71]. In summary, combining GSIs with other drugs for treating breast cancer can maximize the efficacy of various medications, increase success rates, and improve patients' quality of life.

Currently, the screening and development of GSIs from natural products show great promise, offering a new path for discovering and developing novel anti-tumor drugs [72]. Cimigenoside is a monomeric compound extracted from the Cimicifuga plant, demonstrating significant anti-tumor activity. Studies have shown that Cimigenoside can hinder the proliferation and metastasis of human breast cancer cells by blocking the γ-secretase/Notch axis [73].Of course, further research and clinical trials are needed to verify its safety and effectiveness.

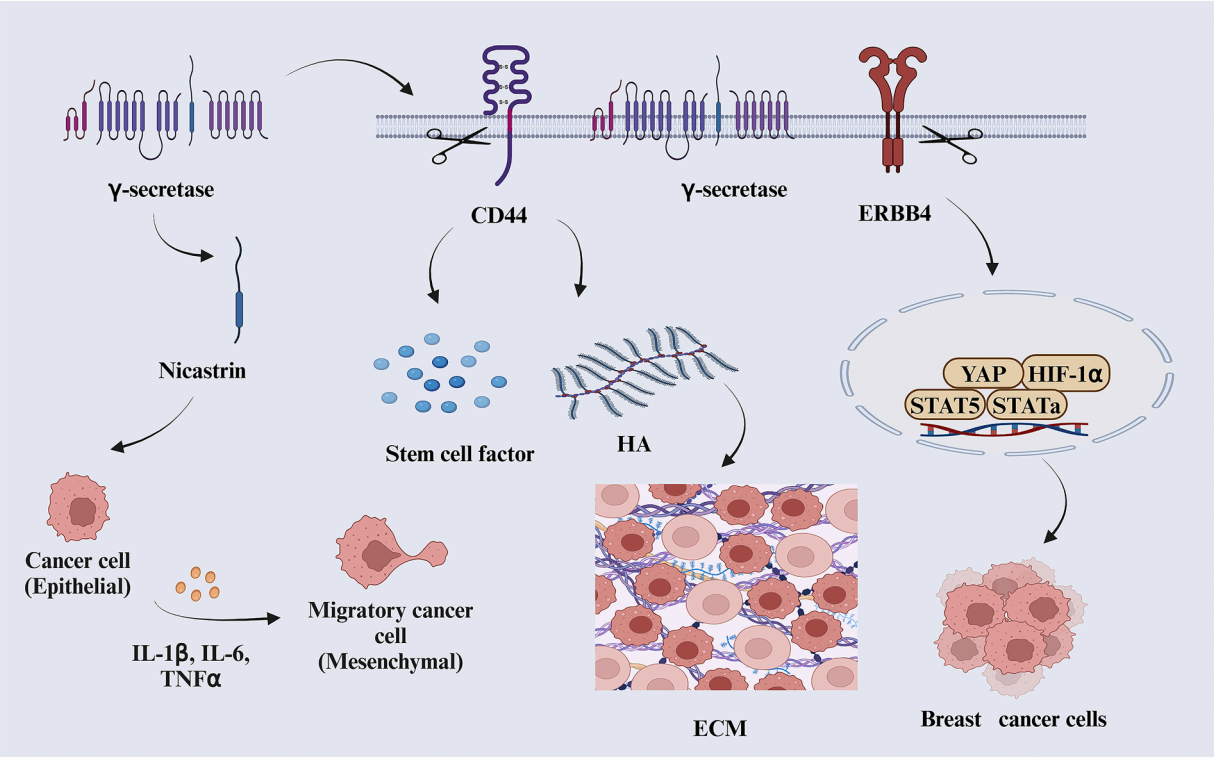

Fig. 8. Overexpression of NCSTN leads to EMT in breast cells, promoting infiltration and metastasis of breast cells. γ-secretase affects the proliferation and differentiation of breast cancer cells by cleaving CD44 protein and ERBB4.

Positive cascade regulation between Furin protease, ADAM, and γ-secretase

The Furin protease, ADAM, and γ-secretase are upstream, midstream, and downstream molecules of the Notch pathway, respectively. The different cellular locations of these three enzymes indicate their interaction and mutual regulation. Current research shows that Furin protease is involved in the maturation process of the Notch receptor. Furin protease is located in the Golgi apparatus, and its main function is to cleave the Notch precursor protein, catalyzing the maturation of the Notch receptor. ADAM protease is involved in the extracellular domain dissociation of the Notch receptor, promoting the activation of the Notch signal. ADAM is located on the cell membrane and releases active NECD by cleaving the Notch receptor. γ-secretase is responsible for further cleaving and releasing NICD, allowing it to enter the nucleus to regulate transcriptional activities [74]. These three proteases form a cascading signal transduction network.

Activation of the Furin protease can enhance the activity of ADAM and γ-secretase, thereby activating and promoting signal transduction of the Notch signaling pathway [75]. Notch-1 directly regulates the expression of Furin protease and increases its activity, thereby enhancing the activity of Membrane type1-matrix metalloproteinase and ADAM10 without affecting ADAM17. This positive feedback loop can amplify Notch-1 signaling, promoting tumor growth and progression [76]. Another study demonstrates that Furin protease, ADAM10, and γ-secretase are involved in the cleavage of the receptor protein tyrosine phosphatase (RPTP). Furin protease and ADAM10 cleave the extracellular domain of RPTP to release a soluble fragment, which activates γ-secretase to further cleaves RPTP. The cleaved extracellular domain of RPTP also activates Furin protease and ADAM10 to create a positive feedback loop that in turn enhances the cleavage and activity of RPTP. This feedback loop plays a crucial role in regulating the transcriptional activity of β-catenin [77]. Therefore, there is a positive feedback regulation between these enzymes. The activation of the Furin protease increases the activities of ADAM and γ-secretase, which help to maintain the function of the Notch signaling pathway. This positive regulatory mechanism provides necessary support for the activation and signal transduction of the Notch signaling pathway, offering new research directions and targets for breast cancer therapy. In terms of drug resistance, abnormal activation of the Notch signaling pathway is associated with chemotherapy resistance in breast cancer cells. Regulating this pathway can overcome the chemotherapy resistance dilemma in clinical treatment of breast cancer [78].

Potential targets related to breast cancer treatment in the Notch pathway

Glycosylation is a biochemical process in which glucose molecules are attached to proteins or other biological molecules. This process regulates cellular signaling, protein stability, cell adhesion, recognition, and other essential life activities. In the extracellular domain of the Notch receptor, there are a series of repetitive EGF domains that often undergo glycosylation modifications. The glycosylation modifications of the Notch receptor mainly include two forms: O-glucosylation, which is catalyzed by O-glucose transferase (Poglut), and O-fucosylation, which is catalyzed by O-fucose transferase (Pofut) [79], as shown in Fig. 9A. Recent studies have shown that Notch receptors lacking O-fucosylation modification are unable to properly bind with their ligands, leading to dysregulation of the Notch signaling pathway (abnormal activation or inhibition) and disrupting Notch signal transduction [80]. Interestingly,

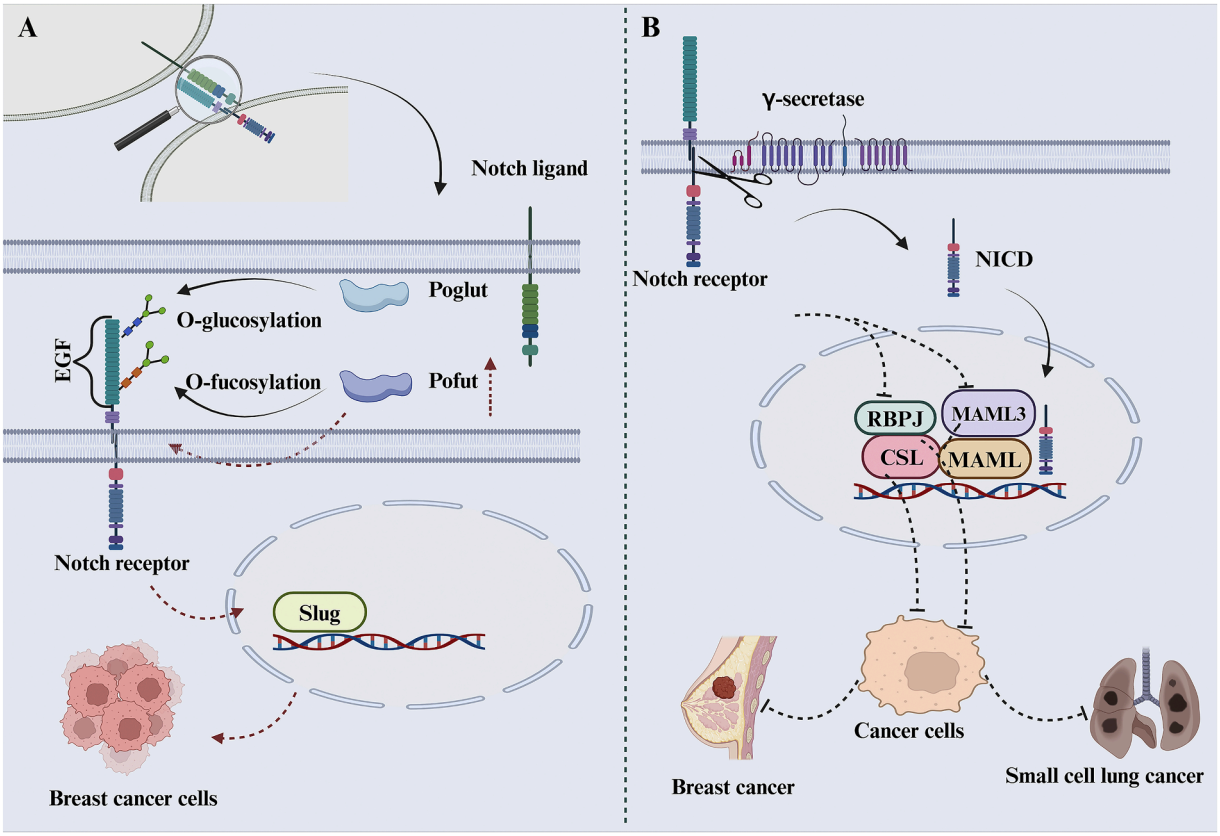

Fig. 9. Glycosylation & Transcription factors. A: Glycosylation modifications of Notch receptors mainly include O-glucosylation and O-fucosylation. Overexpression of O-fucosyltransferase (POFUT) can lead to abnormal activation of the Notch signaling pathway, affecting the invasion and metastasis capabilities of breast cancer cells. B: CSL plays a role in inhibiting tumor growth in breast cancer. Inhibiting the activity of RBPJ and MAML3 can reduce the proliferation and invasion abilities of small cell lung cancer.

overexpression of POFUT1 can enhance the binding of the Notch-1 receptor to Notch ligands, thereby activating the Notch1 signaling pathway. The activated Notch-1 signaling pathway can trigger epithelial-mesenchymal transition (EMT) by controlling the expression of the transcription factor Slug, consequently boosting the invasive and migratory capabilities of breast cancer cells [81]. Therefore, targeted therapy against POFUT1 may be a promising strategy for the treatment of colorectal cancer. In summary, in the glycosylation process of the Notch pathway, O-fucose transferase can be considered a potential target in the Notch pathway, with significant implications for cancer treatment and research.

The transcription factor in the Notch pathway may serve as another key target for cancer. The MAML protein is a transcription co-activator that plays a crucial role in Notch signaling. It interacts with the NICD and the transcription factor CSL to form an active Notch transcription complex, thereby activating the target genes of the Notch pathway. Studies have shown that the expression of MAML protein is closely related to the activity of the Notch signal. In certain tumors, the overexpression or mutation of the MAML protein may lead to abnormal activation of the Notch signal, thereby promoting the occurrence and development of tumors [82]. Similarly, the transcription factor CSL also plays a crucial role in the Notch pathway, as shown in Fig. 9B. In breast cancer cells, the loss of CSL can promotes tumor growth and sensitivity to low-oxygen environments. Research has found that the mice received the transplantation of breast cancer cells lacking CSL exhibit accelerated tumor growth and increased tumor volume [83]. In prostate cancer and breast cancer cells, knocking down CSL expression using lentivirus-based shRNA can decrease CSL's DNA binding activity, resulting in the suppression of cell proliferation [84]. Recombinant signal binding protein RBPJ (a member of the CSL family) and MAML3 (Mastermind-like 3) are potential therapeutic targets for small cell lung cancer. Research has shown that inhibiting the activity of RBPJ and MAML3 can reduce the proliferation and invasion capabilities of small cell lung cancer and decrease the expression of matrix metalloproteinase [85]. The aforementioned studies suggest that inhibiting the function of MAML or CSL can suppress the proliferation and metastasis of tumor cells, rendering them potential targets for cancer treatment. In the future, with a deeper understanding of the Notch pathway and MAML, CSL, therapeutic strategies targeting MAML and CSL, such as small molecule inhibitors and antibody drugs, can be developed to intervene in the abnormal activation of the Notch pathway.

Outlook

In the treatment of breast cancer, Furin protease, ADAM metalloprotease, and γ-secretase all influence the proliferation, migration, and invasion of tumor cells. Furin protease converts the precursor protein of the Notch receptor into its active form, which is a crucial step to initiate Notch signal transduction, impacting the proliferation, survival, and invasion ability of breast cancer cells. Moreover, Furin protease participates in signaling pathways that involve the growth and invasion of breast cancer cells, such as VEGF (vascular endothelial growth factor) and TGF-β (transforming growth factor-β) [86]. Due to its critical role in tumor progression, Furin protease is receiving attention as a potential therapeutic target. Inhibitors targeting Furin protease are currently being developed and may become a new strategy for treating breast cancer. Some members of the ADAM metalloprotease family are also involved in regulating the Notch pathway, affecting signal transduction processes through cleavage and activation of Notch receptors. In breast cancer, ADAM metalloproteases participate in tumor cell growth, invasion, and metastasis by modulating the Notch signaling pathway. Overexpression of certain ADAM members is closely associated with the malignancy of breast cancer. Therefore, inhibitors targeting ADAM metalloproteases may be promising potential drugs for treating breast cancer metastasis. γ-secretase is another key enzyme that influences the Notch signaling pathway, participating in the final cleavage of Notch receptors, affecting the ultimate activation of the pathway, and playing an important role in the proliferation of BCSCs and regulation of the tumor microenvironment. Inhibiting γ-secretase can affect the proliferation and invasion capabilities of breast cancer cells, making it one of the potential targets for treating breast cancer. Furin protease, ADAM metalloproteases, and γ-secretase are of significant importance in breast cancer treatment, offering potential for novel therapeutic strategies for breast cancer.

The combination of Furin protease with nanomaterials holds great promise in the treatment of breast cancer. Recently, researchers have reported a tumor-specific Furin protease-catalyzed nanoprobe Fe_3O_4@Gd-DOTA NPs (FFG NPs) for the treatment of malignant breast cancer. This nanoprobe can provide both molecular magnetic resonance imaging (mMRI) and structural magnetic resonance imaging (sMRI) information. The ratio of mMRI to sMRI signal intensity can be used for the differential diagnosis of malignant breast cancer [87]. A research team has utilized gold nanoparticles (AuNPs) induced by the Furin protease to treat breast cancer. These nanoparticles can be recognized and aggregated by Furin in tumor cells, increasing the accumulation of drugs within the tumor. The researchers loaded the chemotherapy drugs doxorubicin (DOX) and hydroxychloroquine (HCQ) onto these aggregated AuNPs and discovered that the most effective therapeutic outcome was achieved when the mass ratio of DOX to HCQ was 1:6 [88]. This approach overcomes the drug resistance of breast cancer to chemotherapy drugs, showcasing the potential of combining Furin protease with nanoparticles in tumor treatment.

Developing specific inhibitors for ADAM metalloproteases is a potential therapeutic strategy. Currently, there are several ADAM17 inhibitors under development. The D1(A12) monoclonal antibody can directly inhibit the activity of ADAM17. D1(A12) has been shown to significantly reduce the survival and migration abilities of TNBC cells by promoting cell death [89]. In addition, some small molecule ADAM17 inhibitors such as PF-548 are also actively being developed, aiming to interfere with the growth and metastasis of tumor cells by inhibiting the activity of ADAM17 [90]. Similarly, GI254023X is a selective inhibitor of ADAM10, this inhibitor can effectively reduce the invasive and migratory abilities of breast cancer cells [52]. These studies indicate that inhibitors targeting ADAM metalloproteases have important potential applications in the treatment of breast cancer.

γ-secretase modulators have shown great potential in the treatment of breast cancer. Sulindac sulfide is a γ-secretase modulator that inhibits the activity of the Notch pathway, thereby suppressing the growth and spread of breast cancer cells. Additionally, Sulindac sulfide can enhance the effectiveness of immunotherapy and increase the sensitivity of breast cancer patients to PD-1 immunotherapy [91]. γ-secretase modulators as potential options for breast cancer treatment, are expected to address the limitations of current clinical treatment methods. By inhibiting the Notch signaling pathway, these modulators can impact tumor cell proliferation, metastasis, and immune suppression, thereby offering new treatment prospects for breast cancer patients.

In conclusion, Furin protease, ADAM metalloprotease, and γ-secretase are key enzymes in the Notch pathway and important targets for breast cancer. Although new therapeutic strategies targeting them, including inhibitors and antibodies, have been developed, these strategies still require further clinical validation. In the future, attention should also be paid to other potential targets in the Notch pathway, such as glycosylation processes and transcription factors, to provide more possibilities for the treatment of TNBC.

Ethical approval

Not applicable.

CRediT authorship contribution statement

Kuo Yao: Writing – review & editing, Writing – original draft. **Xiang-**

Yi Zhan: Writing – review & editing, Supervision. **Mei Feng:** Visualization, Investigation. **Ke-Fan Yang:** Visualization, Investigation. **Ming-Sheng Zhou:** Writing – review & editing, Supervision, Conceptualization. **Hui Jia:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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