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Current Drug Targets, XXXX, *XX*, 1-9

REVIEW ARTICLE

Role of γ-Secretase Inhibitors for the Treatment of Diverse Disease Conditions through Inhibition of Notch Signaling Pathway

Arijit Mondal[1,*], Sankhadip Bose[2], Sabyasachi Banerjee[3] and Dilipkumar Pal[4]

[1]*Department of Pharmaceutical Chemistry, Bengal College of Pharmaceutical Technology, Dubrajpur-731 123, India;* [2]*Department of Pharmacognosy, Bengal School of Technology, Hooghly- 712102, India;* [3]*Gupta College of Technological Sciences, Asansol-713301, India;* [4]*Department of Pharmaceutical Sciences, Guru Ghasidash Vishwavidyalaya, Bilaspur- 495009, India*

ARTICLE HISTORY

Received: August 28, 2020
Revised: December 17, 2020
Accepted: February 09, 2021

DOI:
10.2174/1389450122666210515161312

Abstract: γ-secretase is an intramembrane protease sub-assembly that sunders transmembrane proteins. It is involved in intramembrane proteolysis and also contributes to the regeneration of transmembrane protein. The Amyloid Precursor Proteins (APPs) are typical γ-secretase substrates. These proteins are cleaved to produce 36-43 amyloid-beta (Aβ) amino acid peptides. Abnormal folding of these proteins fragments leads to amyloid plaques, which are frequently encountered in Alzheimer's disease. Some Type I class of integral membrane proteins is processed under the influence of γ-secretase, such as receptor tyrosine-protein kinase erbB4 and CD44 glycoprotein. γ-Secretase is being explored in several diseases as a clinical goal. Both γ-secretase inhibitors (GSIs) and γ-secretase modulators (GSMs) are being evaluated for this purpose. A large amount of γ-secretase inhibitors (GSIs) from peptide to non-peptide have been disclosed, offering several lead compounds for the design and optimization of γ-secretase targets, but most GSIs lack sufficient potency, exhibit low penetration in the brain, and manifest low selectiveness. γ-secretase inhibitors are obliquely a regulator of a γ-secretase substrate Notch, and valuable in the development of β-amyloid peptide (Aβ). These γ-secretase inhibitors block the Notch signaling pathway in autoimmune and lymphoproliferative disorders, like Autoimmune Lymphoproliferative Syndrome (ALPS) and systemic lupus erythematosus (SLE), and perhaps even in cancerous cell proliferation, angiogenesis, and cellular differentiation of human-induced pluripotent stem cells (hiPSC). The current review portrays the mechanism, regulation, and inhibition of γ-secretase in the management of a wide assortment of diseases.

Keywords: Amyloid β-protein precursor, γ-secretase inhibitors, notch signalling, notch ligands, autoimmune lymphoproliferative syndrome (ALPS), angiogenesis.

1. INTRODUCTION

In combination with three other proteins, anterior pharynx-defective-1 (APH1), presenilin enhancer 2 (PEN2), and nicastrin, presenilin 1 (PSEN1) or presenilin 2 (PSEN2) serves as the catalytic center of γ-secretase [1-3]. This intramembrane protease splits into more than a hundred Type 1 membrane proteins within the transmembrane domains (TMDs) [4]. It has been noted that cleavages catalyzed by γ -secretase regulate certain signaling pathways by modulating the cytoplasmic domain of specific transmembrane proteins, enabling them to relay signals into the nucleus [5, 6]. Regulated intramembrane proteolysis performed by γ-secretase activates the cells to transfer and control signals *via* lipid bilayer, although it can also recycle transmembrane protein in other situations [7, 8]. γ-secretase is a promiscuous protease because of the cleaved transmembrane domain (TMD) sequences. In specific subcellular compartments, the major cytoplasmic determinants of cleavage are usually preceding ectodomain shedding, and the γ-secretase is co-localized with the corresponding membrane stub [9, 10]. γ-secretase initially cleaves a protein's TMD at a location close to the membrane's cytoplasmic border [6, 9-13]. Such initial cleavage is then accompanied in amyloid β protein precursor (AβPP) by cleavages of 3 to 5 consecutive di, tri, or tetrapeptide [12]. Therefore, transmembrane protein γ-secretase cleavage leads to two potentially active parts comprising the cytoplasmic domain and a small secreted peptide. The standard sequencing with γ-secretase of a Type 1 membrane protein is shown in Fig. (**1**).

The initial γ-secretase cleavage site and the degree of the processivity are not specified for most substrates. Full γ-secretase inhibition seems to be an excellent strategy. However, it was found that γ-secretase performs a larger biological role and cleaves multiple proteins to yield physiologically essential materials. Therefore, *in-vivo* serious adverse effects stem from complete inhibition [14-16]. γ-secretase comprises four proteins, such as presenilins (presenilin 1 or 2), nicastrin, presenilin enhancer 2 (PEN-2), and anterior pharynx-defective-1 (APH-1) (Fig. **2**) [2].

*Address correspondence to this author at the Department of Pharmaceutical Chemistry, Bengal College of Pharmaceutical Technology, Dubrajpur-731123, India; Tel: +91-967-412-4916;
E-mail: juarijitmondal@gmail.com

1389-4501/XX $65.00+.00

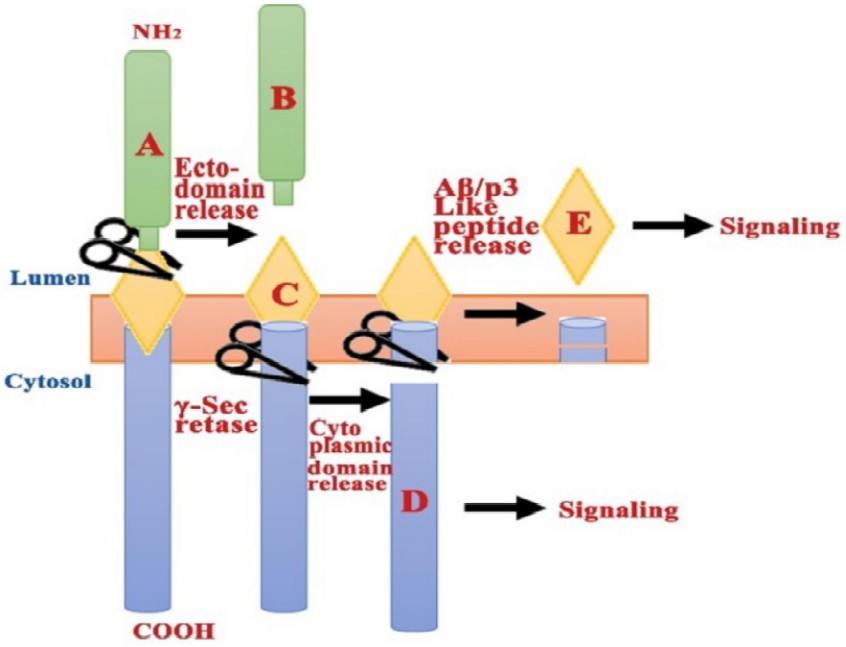

Fig. (1). Stepwise cleavage of γ-secretase. Sequencing of a Type 1 transmembrane protein (**A**), with "sheddase" accompanied by sequential γ-secretase cleavages, leads to the release of many potentially bioactive protein fragments comprising of the ectodomain (**B**), a transmembrane carboxyl terminal fragment (CTF) or stub (**C**), the cytoplasmic domain (**D**) and an Aβ/p3 like domain (**E**). Thus, γ-secretase produces potentially bioactive fragments (**D**) through the initial cleavage and (**E**) through the steps of processed cleavages. GSIs block the initial splitting and GSMs change the processivity. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

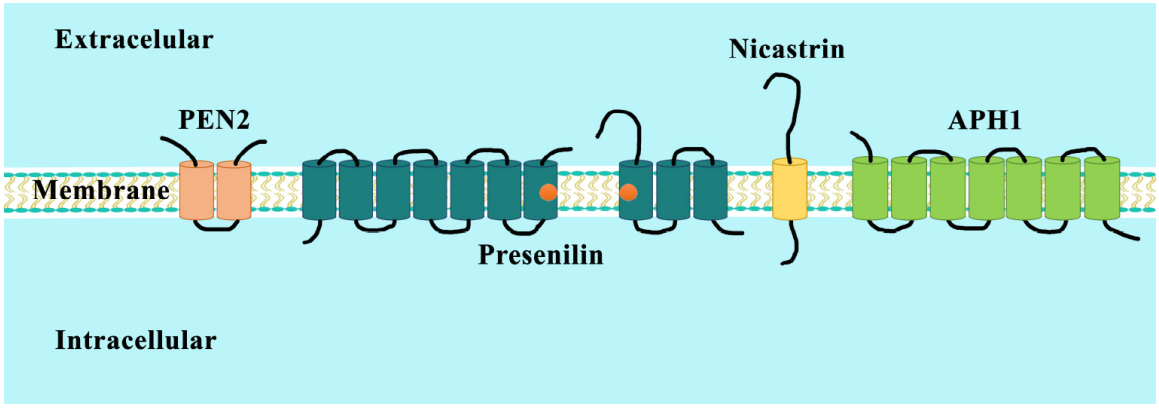

Fig. (2). The structure of the γ-secretase complex. Presenilins are catalytic parts of γ- secretase complexes comprising of proteins, such as presenilin-enhancer-2 (PEN2), nicastrin, and anterior-pharynx defective-1 (APH1). The catalytic activity of the aspartyl residues in transmembrane domains 6 and 7 are marked by orange rings. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Past research on the developmental biology of the inner ear has shown the important function of Notch in determining the cell fate of hair cells or supporting cells from cochlear sensory cells [17-20]. Renal fibrosis is marked by inflammation of the epithelial cells, leukocyte movement, decreased deposition of the Extracellular Matrix (ECM), and the proliferation and activation of myofibroblasts. Mature myofibroblasts are produced in response to kidney damage, including interstitial fibroblasts, and due to the circulating pericytes, Tubular Epithelial Cells (TECs), endothelial cells, and fibrocytes [21]. It is composed of four notch receptors (Notch 1, Notch 2, Notch 3, and Notch 4) and five Notch ligands, such as Delta-like 1 (DLL1), DLL3, and DLL4, and Jagged 1 (JAG1) and Jagged 2 (JAG2) in vertebrates. When a ligand binds, Notch receptors are subjected to the sequence of cleavages facilitated by the γ-secretase, culminating in the secretion of the Notch intracellular domain (NICD). The γ-secretase inhibitor prevents the secretion of

NICD [22]. The current review portrays the mechanism, regulation, and inhibition of γ-secretase in the management of diverse disease conditions.

2. GAMMA-SECRETASE INHIBITORS

A variety of compounds that exhibited significant inhibition of Aβ secretion and elevated expression levels of AβPP carboxyl-terminal fragments (CTFs) generated by α/β-secretase induced ectodomain shedding were reported in the development of Aβ inhibitors between the mid and late 1990s [23-30]. The targeted protease was unknown when the first AβPP-processing compounds were identified, but cleavage activity was named γ-secretase. These inhibitors are referred to as γ-secretase inhibitors (GSIs). γ-secretase cleaves the AβPP in the transmembrane domain which produces numerous Aβ peptides. Many theories described the essence of the behavior and the responsible proteases behind it [31, 32]. In addition, the concept that protease would break peptide bonds usually inside a Transmembrane Domain (TMD) of a protein was generally opposed at that time, sparking more debate about the existence of the protease responsible. Many trials of inhibitors have also shown that γ-secretase has had several biochemically associative cleavage events, suggesting that these could be due to other proteases [33, 34]. γ-secretase having the catalytic center PSEN1 or PSEN2 and the proteins, such as APH1, PEN2, and nicastrin is required for the assembly of the complex and cell stabilization [1-3, 35]. Although it is theoretically feasible for small molecules which block the γ-secretase cleavage to be able to interact with one of the subunits, GSI binding analysis exhibited that GSIs regulate the PSEN1 and 2.

PSEN1 and 2 belong to a bigger family of aspartyl proteases which comprises of five human homologs identified as the signal peptides peptidase (SPP)/HM13 gene, signal peptides peptidase like-3 (SPPL3), signal peptides peptidase like-2A (SPPL2A), signal peptides peptidase like-2B (SPPL2B), and signal peptides peptidase like-2C (SPPL2C) [36-38]. SPPs vary from the PSENs in which the Type 2 transmembrane domain is divided as compared to the Type 1 membrane proteins [36, 39, 40]. The reciprocal sensitivity of cleavage seems to be defined by the contrary alignment of two activating aspartic acid residues within the SPPs and the leaders of the PSEN band. Notably, in the latest crystal configuration of *Methanoculleus marisnigri* (strain JR1), SPP has been identified [41]. This arrangement shows that the catalytic aspartate residues located inside competing transmembrane domains and the lipid membrane surface are close to one another.

Many, but not all, GSIs also block the signals of peptide peptidases, but this is not observed at a systematic level [37, 42]. This is a significant aspect when contemplating biological activities of different GSIs which could affect both biological response and potential toxicity. It is reported that SPP is the primary target goal of GSIs, as SPP level of expression is elevated in most cells than PSEN / γ-secretase [43]. GSIs that control SPPs can, therefore, be effective instruments to analyze the biological effects of SPP inhibition [44-46].

3. NOTCH SIGNALING

The cell-to-cell signaling mechanism controls the self-renewal of the embryonic and adult stem cells. It also regulates the cellular quiescence, cell fate and differentiation, the proliferation of cells, induction of apoptosis, and tumorigenesis. An investigation elucidated that the Notch pathway is the outcome of a gene mutation in the fruit fly *Drosophila ampelophila* that eventually develops a knot or incisions in the fly wings' end [47]. The allele of this fly wing length Notch gene is lin-12 [48, 49]. Finally, the Notch gene is characterized and cloned [50, 51]. The highly conserved Notch signaling system, cellular interactions, and Notch-mediated lateral specification during the embryonic growth phase were further elucidated through associated research using the nematode worm *Caenorhabditis elegans*.

4. DEVELOPMENTAL NOTCH

The Notch pathway of signaling affects cellular fate concerning the processes of lateral inhibition, lineage decisions, and inductive mechanisms [52]. Notch controls early organ development and cell differentiation during embryogenesis through cellular signaling mechanisms. In knockout mice in which the Notch-1 and Notch-2 receptors and DLL1 & JAG1 ligands were knocked out, the importance of the notch signaling pathway was verified [53, 54]. These knockout mice showed serious deficiencies that lead to embryonic or fetal/perinatal death. Within the early embryo's pluripotent stem cells, notch stimulation triggers lateral repression causing a limited percentage of cells to embrace a particular cell fate, and the adjacent cells are barred from cellular differentiation [55]. Similar observations were reported in embryonic chick retinal neurons by over-expressing the Notch Delta 1 receptor, which triggers lateral repression of neighboring progenitor cells by the mechanism of neuronal differentiation [56]. Lastly, Notch facilitates or induces the production of a certain type of cell in inductive cell-cell signaling, usually among specific (non-equivalent) cell populations. Such interactions are essential for establishing boundaries formed by the distinct signaling among cell types. The requirement for inductive cellular signaling is apparent through developmental research. The notch signaling pathway influenced the Spemann organizer through regulation of the dorsal-ventral patterning that affects the growth and pattern of the wing in Drosophila (small fruit flies) [57].

5. NOTCH RECEPTORS

These are single-pass transmembrane glycoproteins and heterodimers found in the cell membrane. There are four mammalian notch receptors known as Notch 1, Notch 2, Notch 3, and Notch 4. Notch-1 is the larger receptor among the Notch receptors and Notch-4 is the smallest one [55]. These consist of extracellular, transmembrane, and intracellular domains (Fig. **2**). The Notch is a polypeptide having a molecular weight above 300 kDa and is produced naturally in the organelle endoplasmic reticulum. Moreover, it is exposed to an *O*-linked glycosylation reaction in which the sugar molecule gets attached to the oxygen atom of serine/threo-

nine residues of the protein. *O*-fucosyl transferase1 regulates the linkage of O-linked fucose with the Notch epidermal growth factor (EGF)-like repeats. The Notch protein precursor is accompanied by the guanosine triphosphate hydrolase (GTPase) Rab (G protein) across the secretory route into the trans-Golgi network (TGN). The O-fucoses-specific β1,3-N acetylglucosaminyl-transferases (β3GnT) enzyme of the Fringe family tends to expand the linkage of O-linked fucose with the carbohydrate sequence on either serine or threonine amino acid residues of the protein. Ligand-mediated activation is controlled by the modulation of the Notch receptor through Fringe proteins. After that, furin-like pro-protein convertase cleaves the protein into N-terminus and C-terminus segments, which are then transferred into the cell's plasma membrane [58, 59]. The segments are integrated inside the cell membrane as a completely active heterodimer protein. This heterodimer receptor non-covalently attaches with the calcium cation and interacts with the Notch ligand. The ligand-binding region of the Notch receptor is located at its N-terminal extracellular domain and is composed of numerous conjunctions of 29-36 EGF-like repeats. Six cysteine residues of the extracellular domain create three bridges of the disulfide intradomain. The functional transmembrane domain is located near the cell membrane, adjacent to the extracellular domain. The transmembrane domain is comprised of RAM23 juxtamembrane negative functional segment and the heterodimer segment, keeping the Notch receptor in an inactivated condition. The Notch receptor also contains a C-terminal intracellular field which extends within the cell membrane up to the cell cytoplasm. The C-terminal intracellular field comprises the following distinct parts: (1) the recombination signal binding protein for immunoglobulin kappa J region [RBP-JK associated molecule or RBP-J kappa-associated module (RAM) domain], associated with nuclear localization sequence (NLS); (2) seven iteration of cdc10/ankyrin repeats; (3) the trans-activating domain (TAD) with an NLS; and (4) polypeptide enriched proline (P), glutamic acid (E), serine (S), and threonine (T)-rich motifs (PEST peptide sequence) with degrons which stabilize Notch intracellular domain inside the nucleus to promote fast proteolytic degradation or proteolysis. The trans-activating domain is identified in Notch-1 and Notch-2 receptors, but this is absent in other Notch receptors.

6. NOTCH LIGANDS

Mammals have five distinct Notch ligands, named Delta-like 1 (DLL-1), DLL-3, and DLL-4, and Jagged-1 (JAG-1), and JAG-2 ligands, as shown in Fig. (**3**) [55, 60]. Notch ligands are Type 1 transmembrane proteins that cross the membrane only once and firmly hold together. They further trigger the trans-interaction of the Notch receptor on the surface of the adjacent cell. The Notch ligands are comprised of extracellular and intracellular domains. The Jagged ligands comprise 6-16 EGF-like repeats in the extracellular domain, because of which they are longer than Delta-like ligands. A cysteine-rich region is placed at the end of the EGF-like repeats. The Jagged ligands have one more cysteine-rich region. The intracellular cytoplasmic tail domain (CTD) is shorter than the extracellular cytoplasmic tail domain. It consists of a PDZ (Post-Synaptic Density Protein [PSD95], Drosophila Discs-large 1 tumor suppressor protein [hDlg/Dlg1], and Zonula Occludens Protein 1 [ZO-1]) specific binding motifs that assist in intracellular protein-protein interactions (PPIs). The cell-surface receptor facilitates two consecutive proteolytic cleavages at the specific cleavage site, resulting in the entry of NICD inside the nucleus to function as the transcriptional activator to increase the gene transcription [61].

Cellular interaction facilitates the notch ligand to bind with the receptor which undergoes limited range, and only one-directional sequence of events starting from the cell membrane for cellular communication and triggering the CSL family transcription factors, such as C promoter binding factor-1 (CBF-1), suppressor of hairless (SuH), and LAG1 in the cell nucleus. Integration of the Notch receptor by the ligand with its high-affinity EGF-like repeats is represented in Fig. (**4**). The ligand-expressing cells undergo trans-endocytosis, creating a mechanism to displace the Notch receptor's extracellular domain from the transmembrane domain. This explains the role of the α-secretase facilitated S2 site cleavage in “A disintegrin and metalloproteinase” family of ADAM17 (ADAM metallopeptidase domain 17 or tumor necrosis factor-III-converting enzyme TACE) and ADAM10, resulting in the proteolytic cleavage of the extracellular region of the transmembrane section of the Notch receptor to produce 12 amino acids. This proteolytic cleavage produces a carboxy-terminal fragment named the Notch extracellular truncation (NEXT) [62]. The ligand-Notch extracellular component triggers trans-endocytosis and endosomal induced degradation or recycling pathway in the ligand--expressing cells. Monoubiquitisation with E3 ubiquitin ligases Mind bomb-1 (Mib-1) and Mib-2 or Neuralized E3 ubiquitin-protein ligase-1 (NEURL1) and NEURL-2 marks the endocytosis ligand.

The remaining part of the Notch extracellular truncation reveals the S3 and S4 splitting points. These cleavage sites are regulated through the γ-secretase-complex. γ-secretase substrates play a significant role in breast cancer. This transmembrane aspartyl proteinase is a large complex, comprising either presenilin 1 (PSEN1) or presenilin 2 (PSEN2) catalytic subunit, a seven-pass transmembrane protein, and integrally associated transmembrane protein nicastrine (NCT), anterior pharynx-defective 1 (APH1), and presenilin enhancer 2 (PEN-2) accessory subunits of two-pass trans-membrane protein. APH1 and nicastrine sustain the PEN-2 inducing endoproteolysis of presenilin [63]. At the moment of receptor activation, E3 ubiquitin ligases Numb/Itch promote the NICD for proteasomal degradation linked to the inner cell membrane. γ-secretase helps the NICD within the cell membrane to enter inside the cytoplasm and ultimately passes to the nucleus (Fig. **5**) [64].

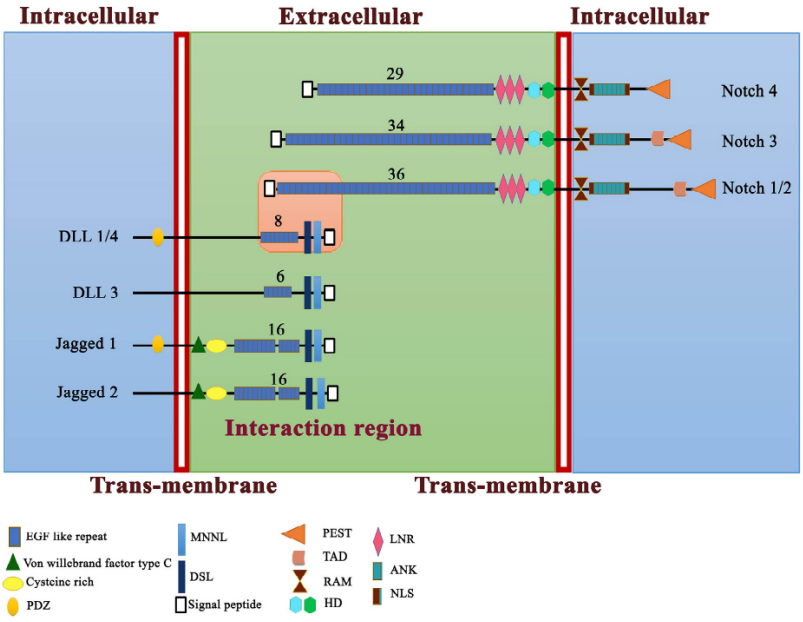

Fig. (3). Notch receptors and ligands. Five Notch ligands (Jagged1, Jagged2, DLL1, DLL3, DLL4) and four Notch receptors (Notch 1, 2, 3, and 4) are available. Notch ligands and Notch receptors contain the extracellular domain, a transmembrane domain, and an intracellular domain. For both ligands and receptors, the extracellular domain is composed of EGF-like repeats, with each receptor and ligand creating separate repeat numbers specified as shown. The position is the same for Notch1 and Notch2. In the DLL4 ligand and Notch1, the interaction region occupies most of the DLL4 extracellular domain. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

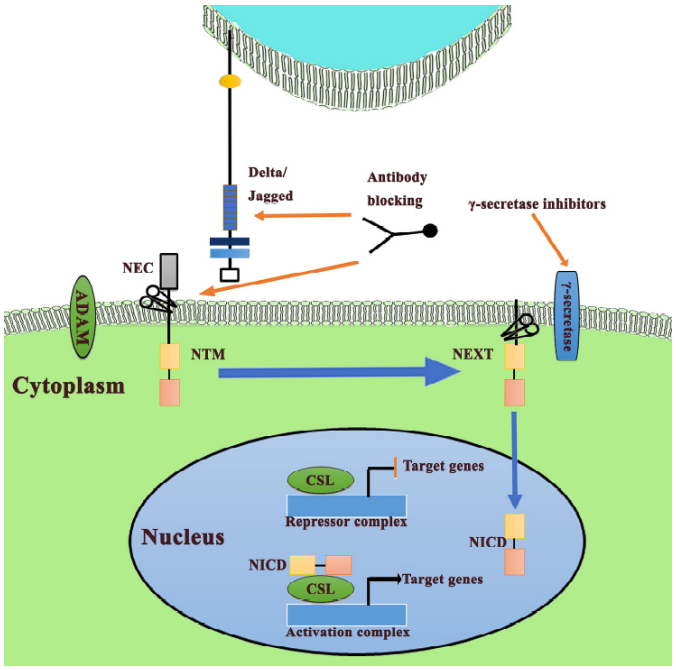

Fig. (4). Notch signaling pathway segments. Ligand binding to the Notch receptor, which is subjected to two-step proteolytic fragmentation by ADAM family proteases and γ-secretase, induces a notch signaling, releasing the NICD. The NICD is transferred inside the nucleus, in which it attaches to CSL and converts the complex from a repressor to a Notch target gene activator. Two main categories of Notch inhibitors could suppress Notch signaling: γ-secretase inhibitors and monoclonal antibodies guided towards Notch receptors and ligands. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

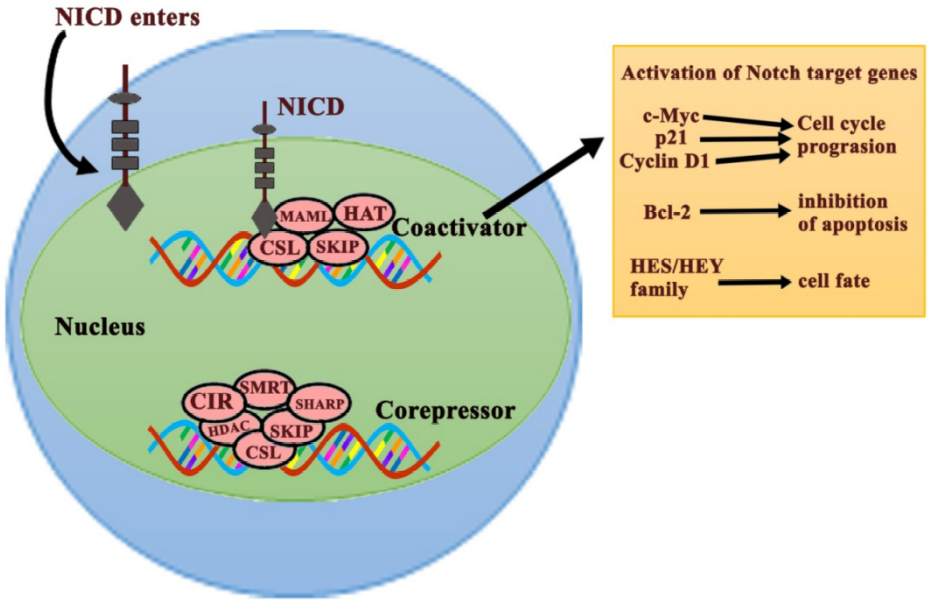

Fig. (5). Notch-mediated nuclear transcription. Abbreviations utilized: NICD, Notch intracellular domain; CIR, CBF-interacting repressor; CSL, C promoter binding factor-1 [CBF-1], suppressor of hairless, Lag-1; HDAC, histone deacetylase; SMRT, silencing mediator of retinoid and thyroid hormone receptor; SHARP, SMRT/HDAC1 associated repressor protein; HAT, histone acetyltransferase; MAML, mastermind--like 1-3; SKIP, ski-interacting protein; Bcl-2, B-cell lymphoma 2; HES, hairy and enhancer of split; HEY, HES related with YRPw motif protein. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

If the Notch intracellular domain ankyrin-repeat motif is linked with the DNA-binding transcription factor CSL Rel-homology region, NICD generates a transcriptional activation protein linked with CSL protein at the nucleus. The entry of transcriptional cofactor co-repressors (CoRs), such as class I or II histone deacetylases (HDACs), CBF-1-interacting corepressor (CIR), SKI-interacting protein (SKIP), silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) mediator, and SMRT / HDAC -1-associated repressor protein (SHARP), and induction of transcription factor co-activators (CoAs) such as mastermind-like protein 1-3 (MamL1-3) results in increased specific gene expression. A few of the targets of the Notch gene which can be triggered usually involve c-Myc (proto-oncogenes), p21 (the cyclin-dependent kinase inhibitor), and cycline D1, B-cell lymphoma 2 inhibiting the action of pro-apoptotic proteins, and Hairy/ enhancer of a split of the basic helix-loop-helix (HES 1, 5, 6, and 7), and Hairy/ enhancer of split related with YRPW motif protein 1 (HEY 1) and HEY 2, and HEY-L (transcriptional repressor) protein band. The NICD activity terminates in the nucleus with phosphorylation initiated by the cycline c-dependent kinase 8 (CDK8). The NICD C-terminal PEST domain is phosphorylated by the glycogen synthase kinase-3β (GSK-3β) and is being attacked using E3 ubiquitin ligase SEL10/FBW7 in the proteasome for poly-ubiquitination [65]. Fig. (**4**) represents a nuclear transcript processed by Notch.

γ-secretase inhibitors inhibit the NICD, blocking an unregulated Notch signaling pathway. A new therapeutic approach was utilized in which the γ-secretase inhibitors (GSIs) block the final cleavage of transmembrane Notch (Notch^{TM}), which would minimize NICD levels. So by regulating this pathway of Notch, γ-secretase inhibitors could provide a better therapeutic approach in diverse disease conditions, specifically cancer. Therefore, down-regulating the Notch signaling pathway with GSIs looks promising therapeutic approach.

7. ROLE OF γ-SECRETASE INHIBITORS IN DIVERSE DISEASE CONDITIONS

7.1. GSIs in Cancer

The NOTCH signaling role in cancer could be because of the crosstalk with other signaling pathways, such as Janus kinase/signal transducers and activators of transcription (JAK/STAT), transforming growth factor-β/decapentaplegic (TGF-β), and Wnt pathways [66]. So, various synthetic compounds, phytoconstituents [67, 68], and marine compounds [69] which modulate various pathways could affect the NOTCH pathway as well. The GSIs are actively repurposed as cancer therapies, based on the assumption that NOTCH signaling suppression in selected cancers is therapeutic. γ-secretase inhibitors, such as BMS-906024 and PF-3084014, are potent NOTCH substrates inhibitors, which exhibits mammospheres. Additionally, it was found that the γ-secretase inhibitor inhibits the Notch3 activation and induces cytotoxicity. It also impedes the proliferation of cancer cells utilizing a panel of human lung cancer cell lines. Restricting the expression of the Notch receptor constitutes a preventive approach. Activating notch involves a proteolytic breakdown of the receptor induced by the γ-secretase protein complex.

MRK-003 inhibits the Notch3 pathway, induces cell apoptosis in the lung carcinoma, inhibits the cancerous cell proliferation, and reduces tumor growth. Treatment with MRK-003 resulted in the reduced phosphorylated Bcl-2 and phosphorylated Bcl-xL expression level and upregulation of cleaved poly (ADP-ribose) polymerase (PARP) expression level [70, 71]. Schott *et al*. (2013) carried out and reported the role of γ-secretase inhibitors in combination therapy utilizing docetaxel on breast carcinoma. Inactivation of the Notch signaling pathway can diminish human breast cancer stem cells (BCSC) using breast tumor graft models and enhance the efficacy of docetaxel. *In vitro* analysis reveals that a synthetic small molecule MK-0752 with a potential antineoplastic activity inhibits γ-secretase by inactivation of the Notch receptor. It also blocks the Notch-intracellular domain (NICD) cleavage, succeeding in nuclear translocation [72]. GSIs and glucocorticoids combined treatment will enhance antileukemic effects and reduce *in vivo* gut toxicity. NOTCH1 pathway inhibition reinstates the auto-upregulation of the glucocorticoid receptor and triggers apoptosis through the initiation of the gene encoding Bcl-2 like apoptosis initiator 11 (BCL2L11) [73].

7.2. GSIs in Kidney Injury

Soni *et al*. showed experimentally that the alteration of the Notch signal pathway by inhibitory agents increases cisplatin activity against several cancer cells. However, it induces the risk of increased kidney injury. The treatment of mice with cisplatin resulted in a rise in Delta-like 1 (DLL1) and Notch1 intracellular domain (NICD) protein expression levels in the kidneys. N-N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine-*t*-butyl ester (DAPT), is a γ-secretase inhibitor that lowers the cisplatin-induced increase in renal NICD over-expression levels. The DAPT also reduces the rate of glomerular filtration and cisplatin-inducing tubular injury. Multi-photon microscopy (MPM) showed pronounced necrosis and peritubular vascular dysfunction in the kidneys of cisplatin-treated mice. However, these adverse effects have been abolished by DAPT. Cisplatin-induced DLL1 / Notch1 signaling was substantiated in the immortalized proximal tubule epithelial cell line (HK-2) isolated from normal adult human kidneys. Small interfering RNA (SiRNA)-induced DLL1 decreased expression and DAPT modulated cisplatin triggered the Notch1 cleavage and cytotoxicity in the immortalized proximal tubule epithelial cell line (HK-2 cells). These results show that the Notch1 signaling pathway regulated by DLL1 leads to acute kidney injury caused by cisplatin [74]. Dibenzazepine (DBZ) is a γ-secretase inhibitor that ameliorates kidney fibrosis by inhibiting the activation of transforming growth factor-beta (TGF-β) / Smad2/3. Dibenzazepine inhibits unilateral ureteral obstruction (UUO) induced renal fibrosis and the expression levels of collagen (1α1/3α1), fibronectin (a glycoprotein), and α-SMA (alpha-smooth muscle actin) [75].

CONCLUSION

From a biochemical standpoint, γ-secretase is a complex enzyme and it is gradually being studied as a therapeutic target. More research is required for the progression of GSIs in the management of diverse diseases (kidney fibrosis, cancer, *etc*). The above discussion about the functionality of GSI from various perspectives suggests that suppressing Notch signaling could be a candidate for the treatment of various cancers, drug-induced renal complications, *etc*. Future research will focus on the influence of other substrates on the effects of GSIs; whether all GSI are biologically beneficial or not will be the major factors in development strategies and also the therapeutic action of different GSIs as a preventive or curative measure for different diseases.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors of the present survey would like to thank all the participants who enrolled in this study.

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