Notch Signaling, γ-Secretase Inhibitors, and Cancer Therapy

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Abstract

The Notch signaling pathway represents a critical component in the molecular circuits that control cell fate during development. Aberrant activation of this pathway contributes to tumorigenesis. The role of Notch in human cancer has been highlighted recently by the presence of activating mutations and amplification of Notch genes in human cancer and by the demonstration that genes in the Notch signaling pathway could be potential therapeutic targets. It has become clear that one of the major therapeutic targets in the Notch pathway are the Notch receptors, in which γ-secretase inhibitors prevent the generation of the oncogenic (intracellular) domain of Notch molecules and suppress the Notch activity. This review article summarizes the biological roles of Notch molecules in cancer development with special emphasis on the promise and challenges in applying γ-secretase inhibitors as a new line of targeted therapeutic agents. [Cancer Res 2007;67(5):1879–82]

Background

The Notch signaling pathway is evolutionarily conserved and the basic molecular players in this pathway are ligands (Delta and Jagged), Notch receptors, and the transcription factors (reviewed in ref. 1). Notch is a transmembrane heterodimeric receptor and there are four distinct members (Notch1 to Notch4) in humans and rodents. In a physiologic condition, binding of the Notch ligand to its receptor initiates Notch signaling by releasing the intracellular domain of the Notch receptor (Notch-IC) through a cascade of proteolytic cleavages by both α-secretase (also called tumor necrosis factor-α–converting enzyme) and γ-secretase (Fig. 1A). The released intracellular Notch-IC then translocates into the nucleus where it modulates gene expression primarily by binding to a ubiquitous transcription factor, CBF1, suppressor of hairless, Lag-1 (CSL). This binding recruits transcription activators to the CSL complex and converts it from a transcriptional repressor into an activator, which turns on several downstream effectors. The physiologic functions of Notch signaling are multifaceted, including maintenance of stem cells, specification of cell fate, and regulation of differentiation in development as well as in oncogenesis (2, 3).

In cancers, molecular genetic alterations, such as chromosomal translocation, point mutations, and chromosomal amplification at the Notch receptor loci, are the known mechanisms for constitutive activation of Notch pathway. Despite the different mechanisms, they all result in increased levels of intracellular Notch-IC. The oncogenic potential of Notch was first discovered in human T-cell acute lymphoblastic leukemia (T-ALL). While Notch1 signaling is essential for normal development of T-cell progenitors (4), constitutive activation of Notch1 signaling due to molecular genetic alterations is associated with T-ALL. For example, interstitial deletions of the extracellular portion of human Notch1 due to (7;9) chromosomal translocation are associated with ~10% of T-ALL cases and activating point mutations of Notch1 are present in ~50% of T-ALL cases (5, 6). Constitutive activation of nuclear factor-κB and formation of T-cell leukemia/lymphoma were observed in a Notch-IC transgenic mouse model (7), which indicates a causal role of Notch activation in T-ALL development. In non–small cell lung cancer, chromosomal translocation (15;19) has been identified in a subset of tumors, and the translocation is thought to elevate Notch3 transcription in tumors (8). In ovarian cancer, Notch3 gene amplification was found to occur in ~19% of tumors, and overexpression of Notch3 was found in more than half of the ovarian serous carcinomas (9). Similarly, Notch signaling activation has been shown in the development of breast cancer. In animal models, constitutively active Notch4 expression causes mammary tumors in mice (10) and Notch1-activating mutations contribute to the development of T-ALL. A recent study further shows that overexpression of activated Notch1 and Notch3 in transgenic mice blocks mammary gland development and induces mouse breast tumors (11). Overexpression of Notch3 is sufficient to induce choroid plexus tumor formation in a mouse model, suggesting a role of Notch3 in the development of certain types of brain tumors (12).

γ-Secretase as a Key Mediator of Notch Signaling

Because the Notch-IC signaling plays an important role in cancer development, it is plausible that targeting the Notch signaling steps, including receptor/ligand binding, release of Notch-IC, interaction of Notch-IC and downstream targets, as well as Notch-IC protein stability, can have antitumor effects (13, 14). Currently, one of the emerging approaches for blocking Notch signaling is to suppress the proteolytic step that leads to the generation of intracellular Notch-IC (Fig. 1B). On ligand binding, Notch receptors undergo a series of programmed proteolytic events, first by α-secretase at the extracellular surface, which leads to liberation of the extracellular fragment, and then by intramembranous cleavage mediated by γ-secretase. Notch-IC is then released from the inner surface of cell membrane and is translocated into nucleus where it activates transcription of the target genes. The proteolytic events in Notch signaling activation are comparable with the processes involving amyloid precursor protein (APP) cleavage (Fig. 1B), in which sequential cleavages by β-secretase and γ-secretase release the amyloid β-peptide (the precursor of amyloid plaques found in the brain of Alzheimer’s disease).

γ-Secretase is a large protease complex and is composed of a catalytic subunit (presenilin-1 or presenilin-2) and accessory subunits (Pen-2, Aph1, and nicastrin). All these subunits contain transmembrane domains and thus they are membrane proteins. The pivotal role of γ-secretase in the Notch activation cascade has been well shown in an elegant knockin experiment, showing that introduction of a single point mutation near the transmembrane cleavage site in Notch1 molecules results in an embryonic lethality in mice, which is similar to the effects observed in Notch1
Figure 1. An overview of Notch signaling and proteolytic processing. Top, Notch receptor is a cell surface protein. Interaction with the Notch ligand, such as Jagged, initiates proteolytic cleavage at the extracellular site by \( \alpha \)-secretase followed by cleavage at the intracellular site by \( \gamma \)-secretase, resulting in the release of Notch-IC from the cytoplasmic side of the cell membrane. Notch-IC is then translocated into the nucleus where it interacts with CSL and recruits coactivators (CoA) to form a transcription-activating complex. Notch-IC can be polyubiquitylated and targeted for degradation in a proteasome-dependent manner. However, the location of degradation is currently not clear. Bottom, the proteolytic events involving Notch receptor and APP are similar. APP is an integral membrane protein and is cleaved at the extracellular site by \( \beta \)-secretase followed by \( \gamma \)-secretase to release A\( \beta \) peptides. A\( \beta \) is the major constituent of amyloid plaques and its accumulation is thought to play a central role in Alzheimer’s disease. APP can be also cleaved by \( \alpha \)-secretase and \( \gamma \)-secretase, which leads to liberation of P3 peptides with unknown functions.
knockout (15). Furthermore, the presenilin-1–deficient and pre-
zenilin-1/presenilin-2 double knockout mice had a marked
decrease in Notch-IC generation (16, 17).

Over the past decades, inhibitors for γ-secretase have been actively
investigated for their potential to block the generation of
Aβ peptide that is associated with Alzheimer’s disease (18). Because
γ-secretase inhibitors are also able to prevent Notch receptor
activation, several forms of γ-secretase inhibitors have been tested
for antitumor effects. First, an original γ-secretase inhibitor, IL-X
(ebz-IL-CHO), was shown to have Notch1-dependent antineoplastic
activity in Ras-transformed fibroblasts. More recently, tripeptide γ-
secretase inhibitor (z-Leu-leu-Ne-C8-CHO) was reported to suppress
tumor growth in cell lines and/or xenografts in mice from
melanoma and Kaposi sarcoma (19). Treatment with dipeptide γ-
secretase inhibitor N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-
phenylglycine t-butyl ester (DAPT) also resulted in a marked re-
duction in medulloblastoma growth and induced G0-G1 cell cycle
arrest and apoptosis in a T-ALL animal model (20, 21). Another
γ-secretase inhibitor, dibenzazepine, has been shown to inhibit
epithelial cell proliferation and induce goblet cell differentiation in
intestinal adenomas in Apc
−/−
(min) mice (22). More recently,
functional inactivation of Notch3 either by tripeptide γ-secretase
inhibitor or Notch3-specific small interfering RNA results in
suppression of cell proliferation and induction of apoptosis in the
tumor cell lines that overexpressed Notch3 but not in those with
minimal amounts of Notch3 expression (9). Furthermore, a phase I
clinical trial for a Notch inhibitor, MK0752 (developed by Merck,
Whitehouse Station, NJ), has been launched for relapsed or
refractory T-ALL patients and advanced breast cancers.1 As
discussed above, Notch signaling and APP metabolism are triggered
by the similar proteolytic process; it is foreseeable that γ-secretase
inhibitors that are currently tested in clinical trials for Alzheimer’s
disease may be applicable to treat neoplastic diseases, especially
those tumors known to harbor constitutive Notch activation.

Besides the evidence of γ-secretase inhibitors in directly inactivating
Notch signaling on cancer cells, γ-secretase inhibitors may also
suppress angiogenesis in solid tumors by interfering in the cross-talk
between the tumor and vasculature through the Notch signaling (23).

The exciting studies summarized above strongly suggest a
potential clinical application of γ-secretase inhibitors in cancer
therapeutics. However, one of the major challenges on the way
toward this goal is the untoward side effects associated with the
inhibitors, especially the cytotoxicity in the gastrointestinal tract
(24), which can be exacerbated by conventional chemotherapeutic
drugs. Therefore, balancing efficacy and toxicity of γ-secretase
inhibitors must be considered in future clinical applications. The
possible mechanisms underlying the unwanted cytotoxicity are
multifactorial. First, Notch signaling pathway is known to widely
participate in cellular physiology in normal tissues, including
hematopoiesis and maintenance of arterial smooth muscle (25);
therefore, it is plausible that inactivation of γ-secretase may lead to
dysfunction of vital organs. Second, it is likely that γ-secretase
inhibitors do not exclusively target the Notch signaling pathways.
This is because γ-secretase has many substrates in addition to
Notch receptors, such as several Notch ligands, ErbB4, syndecan
(an extracellular matrix), and CD44 (1). Additionally, γ-secretase
inhibitors may target proteases other than γ-secretase. As proteases
participate in a wide variety of cellular functions, γ-secretase
inhibitors may have other widespread adverse effects in vivo.
Some of the concerns of the nonselectivity of the inhibitors will be
addressed based on the results of the ongoing clinical trials.
Nevertheless, it may prove possible to identify a therapeutic window,
in which partial inhibition of γ-secretase is sufficient to suppress
Notch signaling in cancer cells, whereas the dosage will not signif-
ically affect the functions in normal tissues. It is thought that
the differential killing between cancer and normal cells can be
exaggerated in treating those tumors with constitutive Notch acti-
vation, in which cancer cells are “addicted” to the Notch signaling.

Implications and Future Directions

The findings discussed above have at least two major biological
and clinical implications. First, like wingless (wnt) and Hedgehog
(shh), the Notch signaling pathway is important in controlling both
developmental processes and tumorigenesis. Tumor cells sabotage
the Notch signaling pathway for tumor initiation and/or progression
through constitutive activation by ways of chromosomal transloca-
tion, point mutation, gene amplification, and other epigenetic
events. Second, recent studies suggest that one of the most
promising targets in inactivating the Notch signaling is γ-secretase
complex, the molecular switch for Notch signaling activation.
Recently, there has been an increased enthusiasm in targeting this
pathway using γ-secretase inhibitors for new cancer therapeutics
because accumulating preclinical studies have shown that
γ-secretase inhibitors hold promise as a new target-based therapy
for those tumors with Notch activation. However, before Notch-
based therapy becomes a reality, future studies should primarily
focus on the issues of target specificity and address the possible side
effects that may affect cancer patients who receive this new
treatment regimen. Furthermore, the clinical promise of γ-secretase
inhibitors in cancer therapeutics depends on careful correlation
studies between the molecular genetic alterations in the Notch gene
(e.g., mutations and gene amplification) and clinical response to
γ-secretase inhibitors. To maximize the therapeutic effects (together
with conventional therapeutics) and minimize the adverse side
effects in cancer patients, it is essential to show the “tumor
dependency” of Notch activation experimentally and clinically.
Despite several challenges on the way, it is expected that in the
coming years, there will be substantial efforts in identifying new
specific γ-secretase inhibitors and in opening new clinical trials to
test the potential of this new line of cancer therapeutic agents.

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