Published in final edited form as:

Semin Cell Dev Biol. 2012 June ; 23(4): 465–472. doi:10.1016/j.semcdb.2012.01.016.

Therapeutic approaches to modulating Notch signaling: Current challenges and future prospects

Casper Groth and Mark E. Fortini*

Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107 USA

Abstract

Dysregulated Notch signaling has been implicated in numerous human diseases, including a broad spectrum of cancers. Mutations in Notch1 are prevalent in T-cell acute lymphoblastic leukemia, and abnormal expression of different human Notch receptors contributes to B-cell tumors as well as cancers of the breast, lung, pancreas, skin, prostate, colon, brain and other tissues. Several γ -secretase inhibitors, small chemical compounds that were initially developed to inhibit the activity of the γ -secretase aspartyl protease in Alzheimer's disease, are now being explored for their potential chemotherapeutic applications in Notch-associated cancers. An alternative approach involves the development of antibodies to inhibit specific Notch receptors, their activating ligands, or other components of the Notch pathway in tumors. Here we review recent progress and current challenges in the use of these strategies to modulate Notch signaling for cancer therapy.

Keywords

Notch; cancer; T-ALL; γ -secretase; inhibitor

1. Introduction

We will soon celebrate the centennial of the discovery of the first *Notch* gene mutation in *Drosophila* in March 1913 [1]. Yet after nearly a century of research on Notch signaling, the use of pharmacological compounds to target Notch activity in clinical settings is still in its infancy. The potential therapeutic value of Notch inhibitors is now well appreciated, given the widespread involvement of Notch in cancers and other developmental disorders. Genetic lesions that directly activate Notch have been detected in some cancers, most notably t(7;9) (q34;q34.3) chromosomal translocations that activate human Notch1 in T-cell acute lymphoblastic leukemia (T-ALL; [2]). Subsequent molecular surveys have established that over 50% of human T-ALL is attributable to mutations that activate Notch1 [3], and that mutated or otherwise upregulated Notch receptors are also associated with breast, prostate, pancreatic, lung, cervical, colon, and a wide range of other cancers (reviewed in [4]). Indeed, elevated Notch activity is a contributing factor along with perturbations in other critical signaling pathways in so many different cancers that therapeutic inhibition of Notch

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*Corresponding author address: Bluemle Life Sciences Building, Room 830A, 233 South 10th Street, Philadelphia, PA 19107 USA, Tel: 215-503-7322, mark.fortini@jefferson.edu.

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signaling is likely to be widely applicable, either alone or in combination with other chemotherapeutic approaches.

2. Discovery and development of γ -secretase inhibitors

Much of the current exploration of drugs to modulate Notch signaling has its origins in Alzheimer's disease research. A pathological hallmark of this disease is the presence in brain tissues of amyloid plaques containing amyloid- β peptides produced by the proteolysis of Amyloid Precursor Protein (APP) at sites within and adjacent to the APP transmembrane domain [5]. The APP intramembrane cleavage is performed by a multiprotein aspartyl protease complex termed γ -secretase, which is composed of the four subunits Presenilin, Nicastrin, Aph-1 and Pen-2, and this complex is also responsible for the intramembrane proteolysis of >100 other identified substrates, including Notch [5] (Figure 1).

The discovery of this proteolytic mechanism spurred tremendous interest in the development of γ -secretase inhibitors (GSIs) that could be used to treat and/or prevent Alzheimer's disease. The first specific, highly potent GSI, the difluoroketone peptidomimetic compound DFK167, was designed to mimic the transition state of aspartyl protease catalysis [6] (Figure 2). Further drug development led to the characterization of additional GSIs, including other transition-state analogs based on hydroxyethylamines (such as L-685,458; [7]), helical peptides [8], and dipeptide analogs (such as Compound E, DAPT, LY-411,575, and LY-450,139/semigacestat; [9–12]; Figure 2). Despite their different chemical structures and modes of action, these GSIs all show relatively high specificity and potency with respect to inhibition of γ -secretase (reviewed in [13]).

Early optimism that these first-generation GSIs might be rapidly deployed in the fight against Alzheimer's disease has been greatly tempered by their failures in animal safety trials and human clinical trials [14,15]. Significant toxicity involving gastrointestinal bleeding and immunosuppression was commonly observed, attributable to GSI interference with Notch signaling [16–18]. Despite these findings, the non-selective γ -secretase inhibitor LY-450,139/semigacestat was advanced to Phase III clinical trials involving Alzheimer's disease patients exhibiting mild-to-moderate cognitive impairment. These trials were halted due to severe gastrointestinal toxicity and immune system defects linked to Notch pathway malfunction, and due to the lack of any apparent beneficial effects on cognitive performance of patients (reviewed in [14,15]). As a result, efforts to treat Alzheimer's disease by targeting γ -secretase have now largely shifted to the design of APP-specific, 'Notch-sparing' GSIs, including non-steroid anti-inflammatory drugs, Gleevac, arylsulfonamides, and other compounds, which exhibit >10-fold up to 3000-fold specificity towards APP relative to Notch (reviewed in [13]).

3. GSIs for Notch-targeted cancer therapeutics: T-ALL and other hematologic malignancies

The off-target effects of first-generation GSIs on Notch signaling immediately suggested that GSIs might prove particularly useful in treating Notch-related cancers. Given the well-documented role of overactive Notch signaling in T-ALL, many of the first studies to explore the potential efficacy of GSI-based cancer treatments focused on T-ALL human cell lines and mouse xenograft models. Extensive characterization of T-ALL cell lines has revealed multiple distinct mutational mechanisms that contribute to aberrant Notch1 activation [3,19,20]. In contrast to the rare cases of T-ALL caused by t(7;9) translocations, which account for <1% of T-ALL, >50% of T-ALL cases involve amino acid substitutions or small in-frame deletions or insertions that activate Notch1. In 40–45% of T-ALL, lesions perturb the negative regulatory region (NRR) domain, leading to ligand-independent

signaling or increased sensitivity to ligand [19]. Other Notch1 mutations cause displacement of the extracellular ADAM cleavage site away from the NRR domain, or shifting of the NRR domain away from the transmembrane domain, in both instances leading to de-regulated ADAM cleavage at the extracellular S2 site [19,20]. A different class of Notch1 mutations results in truncation of the C-terminal PEST domain, leading to increased stability of NICD and hence elevated or prolonged transcriptional activation of Notch1 target genes [3]. Notably, mutations abrogating NRR function are often found together with PEST domain mutations, indicating that T-cell transformation is driven by selection for increasingly high levels of Notch activity. Consistent with this notion, Notch1 mutations have been detected as secondary events in T-cell subclonal populations in T-ALL patients [21].

In an early study, five human T-ALL cell lines were found to undergo G₀/G₁ cell cycle arrest, reduction in cell proliferation, and increased apoptosis following treatment with Compound E [3], findings that were subsequently replicated with additional T-ALL cell lines [22–26]. Specific inhibition of Notch signaling was demonstrated by reduced NICD levels and transcriptional downregulation of Notch1-responsive genes. Compound E was also shown to enhance the sensitivity of T-ALL cell lines to other agents, including dexamethasone and imatinib [25]. Similar effects on G₀/G₁ cell cycle arrest and apoptosis were also observed with the cyclic sulfonamide GSI MRK-003 for three T-ALL cell lines [27].

Complicating matters, these studies also revealed that only a subset of T-ALL cell lines responded positively to GSI treatment. Compound E was effective for only 5 out of 30 T-ALL cell lines, whereas MRK-003 was effective for only 5 out of 20 T-ALL cell lines [3,28]. Mixed results were also reported with rodent xenograft models of T-ALL using various GSIs. The GSI PF-03084014 was found to exert robust antitumor effects in six Notch1-driven T-ALL xenografts, reducing NICD levels and expression of the Notch1 target genes *Hes-1* and *c-Myc* [26], and MRK-003 similarly downregulated Notch signaling, inducing apoptosis and causing complete tumor regression in mouse xenografts of thirteen different human T-ALL lines [29]. However, evaluation of the GSI RO4929097 using a panel of mouse xenografts representing several different human cancers revealed no effect on two T-ALL xenografts or six precursor-B ALL xenografts, although tumor growth delays were observed for other xenografts tested, particularly for osteosarcomas [30]. What is the basis for these disparate outcomes with GSI-based treatment of T-ALL cell lines and xenograft models? One likely factor is that a variety of different GSIs and dosing regimes have been employed in the studies performed to date, making it difficult to compare the results. Indeed, different T-ALL cell lines and xenografts are exquisitely sensitive to different dosing methods. For instance, in one mouse xenograft study with PF-03084014, much stronger antitumor efficacy was observed for a 7-days-on/7-days-off dosing schedule compared to a 3-days-on/4-days-off schedule [26]. In another T-ALL xenograft study comparing seven different MRK-003 dosing regimens, high doses administered on a 3-days-on/4-days-off, weekly, or bimonthly schedule were effective, but moderately lower doses administered on similar schedules were ineffective in conferring antitumor protection [29]. These findings highlight the necessity of carefully evaluating GSI dose levels, dosing schedules, and therapeutic windows to determine the optimal design of animal safety trials and ultimately human clinical trials for candidate GSI drugs.

A second factor contributing to the differential response of T-ALL tumors to GSI treatment is that these tumors are genetically heterogeneous. Several T-ALL cell lines that exhibit high levels of NICD yet are resistant to GSI treatment were found to harbor mutations in the *FBW7* gene, which encodes an F-box ubiquitin ligase required for NICD degradation by the proteasome [28]. The *FBW7* lesions altered conserved arginine residues in the WD40

propeller domain, abrogating binding of FBW7 to its substrates and thus allowing NICD to evade its normal downmodulation. A significant percentage (8.1%) of primary T-ALL isolates were also found to harbor *FBW7* mutations, again illustrating the tendency of T-ALL cells to acquire secondary mutations under selective pressure for continued tumor growth. In a microarray screen for gene expression changes associated with GSI resistance, mutational inactivation of the *PTEN* tumor suppressor gene was also documented for multiple T-ALL cell lines [31]. *PTEN* was inactivated in ~8% of >100 cases of T-ALL examined, resulting in hyperactive PI3K/Akt/mTor signaling that confers GSI resistance by bypassing the requirement for Notch signaling during leukemic clone growth. As these studies indicate, it will be of paramount importance to identify suitable biomarkers and perform tumor genotyping to predict which molecular subtypes of TALL and other cancers will respond most favorably to GSI-based therapies.

As with the initial evaluations of GSIs for treatment of Alzheimer's disease, studies involving GSIs and T-ALL found that this approach to treating Notch-linked cancers will face challenges caused by the systemic toxicity of GSI compounds, especially cytotoxic effects on the gastrointestinal tract. Chronic inhibition of Notch signaling in the gut causes metaplastic conversion of proliferative cells in intestinal crypts and adenomas into mucus-secreting goblet cells, resulting in dose-limiting toxicity [17,18,32]. The preclinical T-ALL studies indicate that intermittent dosing regimens are most likely to offer significant therapeutic benefits while minimizing the potential toxic side effects. A non-randomized Phase I clinical trial was undertaken to evaluate the efficacy of the GSI MK-0752 in relapsed T-ALL patients, but yielded unfavorable outcomes including very low antitumor activity and unacceptably high gastrointestinal toxicity [33]. Nevertheless, several new clinical trials are now underway to evaluate GSI-based T-ALL therapies (Table 1).

Emerging evidence indicates that GSIs might also prove useful for treating hematologic malignancies of B-cell origin. High levels of Notch and its ligands have been reported for several B-cell tumors, including B-cell chronic lymphocytic leukemia, multiple myeloma, and Hodgkin's disease [34–37]. The role of Notch signaling in various B-cell neoplasias is complex, with Notch activation reported to cause growth arrest and/or apoptosis in some tumor cell lines derived from precursor-B ALL, Hodgkin's lymphoma, and multiple myeloma neoplasias [37–40], but increased proliferation in other B-cells derived from B-cell chronic lymphocytic leukemia, Hodgkin's lymphoma, and multiple myeloma [34–36]. Moreover, interactions between B cells and the bone marrow stromal microenvironment influence Notch signaling in both the tumor cells and surrounding stromal cells, leading to changes in growth factor secretion, apoptosis, and proliferation that affect tumor growth [37,41,42].

Many B-cell tumor lines have been found to be sensitive to GSIs, providing a rationale for further testing in animal models and ultimately human clinical trials. DAPT treatment causes a significant reduction in cell proliferation for multiple myeloma and Hodgkin's lymphoma cells [36], and GSI-I, -XII, and DAPT induce apoptosis and inhibit growth of Burkitt's lymphoma and large B-cell lymphoma cells [24,43]. Contradictory results have been obtained with precursor-B ALL cell lines, with one study reporting no significant effect of RO4929097 on six different cell lines [30], while another study found that GSI-I induces apoptosis in precursor-B ALL cell lines and primary lymphoblasts, and blocked or delayed engraftment in 50% of precursor-B ALL mouse xenografts [44]. A complicating issue is that unlike truly γ -secretase-specific GSIs, some GSI compounds including GSI-I and -XII also target the proteasome, leading to the suggestion that simultaneous inhibition of both γ -secretase and the proteasome is required for the pro-apoptotic activities of some GSIs in B-cell neoplasias [44]. Alternatively, the GSI-associated cytotoxicity might primarily reflect

targeting of the proteasome rather than γ -secretase, as deduced from a study utilizing GSI-I to treat breast cancer cells [45].

Finally, GSIs also modulate the Notch-dependent interactions between B cells and stromal fibroblasts, osteoblasts, and osteoclasts. GSI treatment ameliorates the stromal cell-mediated drug resistance of multiple myeloma cells *in vitro* [37] and enhances the antitumor activities of doxorubicin and melphalan in a murine multiple myeloma model [46]. Inhibition of Notch signaling by the GSI RH02015SC in a multiple myeloma cell-osteoclast co-culture assay blocked osteoclast activation and led to decreased proliferation of multiple myeloma cells [42]. Thus despite the complexities of Notch signaling and its crosstalk with other pathways in B-cell neoplasias, GSI-based treatments might ultimately prove broadly applicable for this class of hematologic cancers.

4. GSI-based therapies for breast and lung cancer

Notch signaling also plays an important role in many human breast and lung cancers. Indeed, the murine Notch4 orthologue was initially identified by virtue of the fact that it was truncated and constitutively activated by mouse mammary tumor virus (MMTV) insertions [47,48]. Similarly, MMTV insertions into the murine Notch1 orthologue also promote breast tissue tumorigenesis through expression of truncated Notch1 proteins [49]. A novel truncated form of human Notch4 consisting of a portion of the Notch4 intracellular domain is expressed in some human breast, colon, and lung cancer lines [50], and targeted expression of the Notch1 NICD fragment under control of the MMTV long terminal repeat results in breast adenocarcinomas in a mouse model [51]. Even in the absence of such activating lesions, Notch1 and Notch4 are upregulated in some breast cancer samples, which is associated with increased tumor aggressiveness and poorer clinical prognosis [52–55]. Tumor samples expressing high levels of both Notch1 and its ligand Jagged1 are associated with particularly low overall patient survival rates, suggesting a synergistic effect of these expression level changes on tumor progression [53]. Consistent with these findings, GSI treatment of Notch-induced breast cancer cells *in vitro* reduces their ability to proliferate [56] and inhibits metastasis of mammary tumors by blocking the cooperative effects of Notch and TGF β signaling during the epithelial-mesenchymal transition [57]. Animal safety and human clinical trials are currently underway to evaluate the effectiveness of various GSIs for treating breast cancer (Table 1).

Some types of lung cancer might also prove amenable to GSI-based inhibition of Notch signaling. While Notch appears to function as a tumor suppressor in small cell lung carcinoma, Notch activation instead promotes tumor growth in the more prevalent non-small cell lung carcinoma (NSCLC) [58,59]. A balanced translocation involving chromosome 19 has been identified in a NSCLC patient that results in abnormally high Notch3 expression [60], and overexpression of Notch3 with concomitant upregulation of Notch target genes of the *HES* family was detected in ~40% of NSCLC samples [58,60,61]. In a transgenic mouse model, constitutive activation of Notch3 inhibits differentiation of lung epithelial cells, resembling certain aspects of NSCLC tumorigenesis [62]. Based on these observations, the GSI MRK-003 was tested for *in vitro* effects on lung cancer lines and *in vivo* effects on mouse xenografts. Promisingly, MRK-003 treatment inhibited Notch3 signaling as measured by *HES* target gene levels, elevated levels of apoptosis, reduced tumor cell proliferation, and increased tumor dependence on exogenous growth factors in two out of three xenograft models [63].

5. GSI-based treatments for other Notch-related cancers

In addition to hematologic, breast, and lung cancers, Notch has also been implicated in a host of other cancers, including pancreatic, prostate, colon, renal, hepatocellular, cervical,

skin, head and neck, thyroid, and gastric cancers as well as glioblastoma (for comprehensive reviews, see [4,64]). Although the mechanisms by which Notch contributes to these neoplasias is beyond the scope of this review, it is evident that re-expression and activation of the Notch pathway is a common step in the initiation and/or progression of a diverse array of tumors. Numerous studies utilizing human cancer cell lines and animal models have recently established the potential utility of GSI-based treatments for many different cancers, including Kaposi's sarcoma, hepatoma, pancreatic cancer, myeloma, Wilm's tumor, Ewing's sarcoma, medulloblastoma, glioblastoma, osteosarcoma, and colon cancer [30,46,65–69], and GSI-based Notch inhibition is now being evaluated for many of these cancers in preclinical and clinical trials that are currently underway (Table 1).

6. Combinatorial therapies involving GSI compounds with other chemotherapy agents

Given that dysregulated Notch signaling plays an ancillary role in many cancers that are primarily caused by malfunction of other signaling pathways and cell growth mechanisms, a promising approach is to combine GSI-based inhibition of Notch with other chemotherapeutic agents that target these other pathways. In T-ALL, for example, relapsed glucocorticoid-resistant tumor cell lines treated with GSIs regained sensitivity to dexamethasone, although synergistic effects were not observed when GSIs were combined with etoposide, methotrexate, vincristine, or L-asparaginase [25,70]. In T-ALL lines harboring both *Notch1* mutations and *Ab11* fusions, certain combinatorial treatment regimens using GSIs with the kinase inhibitor imatinib also demonstrated synergistic antitumor effects [25]. Similarly, GSIs such as LY-411,575 and MRK-003 were found to prevent or reduce ErbB-2-positive breast cancer recurrence when combined with trastuzumab or lapatinib in breast cancer xenografts, and partially reversed trastuzumab resistance in refractory tumors [71]. Trastuzumab-induced inhibition of ErbB-2 leads to Notch1 activation [72], which in turn activates PI3K/AKT/mTOR signaling [73,74], a tumor-promoting event that is attenuated by GSI treatment of some ErbB-2-positive breast cancer lines [72,75].

The validity of combining GSIs with conventional chemotherapeutic agents has also been confirmed for other cancer types. Oxaliplatin-induced activation of Notch1 signaling in metastatic colon cancer is reduced by simultaneous GSI treatment, resulting in enhanced tumor sensitivity to oxaliplatin [76]. In multiple myeloma, combined inhibition of Notch using GSI-XII treatment and Bcl-2/Bcl-xL using the small molecule ABT-737 resulted in synergistic cytotoxicity in myeloma cell lines and mouse xenograft models [77]. Enhanced antimyeloma effects have also been observed for combinations involving GSI-XII and the widely used chemotherapy drug bortezomib in multiple myeloma cell lines and primary bone marrow isolates [78]. Synergistic antitumor effects have also been documented recently for the GSI MRK-003 together with rapamycin in pancreatic cancer, which was attributed to enhanced inhibition of the PI3K/Akt/mTOR pathway by the combined drug treatment [79].

7. Notch immunotherapy

An alternative to GSI-based approaches for inhibiting Notch signaling is immunotherapy using antibodies directed against Notch, its Delta/Jagged ligands, or other components of the pathway (Figure 1). This strategy offers the advantage of allowing specific members of the pathway to be targeted, potentially minimizing side effects caused by global inhibition of Notch signaling. For example, in cancers where a particular mutated or otherwise dysregulated Notch paralogue is known to be the primary oncogenic culprit, such as Notch1 in T-ALL or Notch3 in certain lung cancers, antibodies that specifically inhibit the relevant

Notch receptor could be used to selectively inhibit dysregulated Notch signaling in tumor cells while hopefully sparing many other, physiologically important Notch activities. Furthermore, if therapeutic antibodies can be delivered through the bloodstream or by other means, they might reach their desired site of action in a more targeted fashion than GSIs, potentially avoiding the gastrointestinal toxicity and other problems associated with GSIs, which were designed to be administered orally and readily cross the blood-brain barrier for the treatment of Alzheimer's disease.

Antibodies have been developed that specifically antagonize the Notch paralogues Notch1, 2, and 3 by recognizing and stabilizing the extracellular negative regulatory region (NRR) of Notch that undergoes a conformational change upon ligand binding to facilitate ADAM protease cleavage [80–82]. These antibodies inhibit signaling from their specific targeted Notch receptors at low antibody concentrations, and Notch1 NRR (NRR1) antibodies effectively inhibit signaling from Notch1 receptors bearing the most common class of NRR mutations found in T-ALL [82]. Notch1-specific and Notch3-specific antibodies have also been developed that bind to the extracellular ligand-binding domain and compete with ligand binding; these antibodies specifically antagonize ligand-activated signaling but require considerably higher antibody concentrations to exert inhibitory effects [80,82]. *In vitro* studies on human tumor lines indicate that these antibodies are able to inhibit oncogenic Notch signaling, albeit not as potently as cell-penetrating, small molecule GSI compounds [82]. Several Notch-targeting antibodies are now being evaluated in preclinical studies, and the anti-Notch monoclonal antibody OMP-59R5 is in Phase I clinical trials (Table 1).

Inhibitory antibodies directed against Notch ligands including Dll1 and Dll4 have also been generated [83,84], some of which are now being evaluated in Phase I clinical trials (Table 1) following *in vitro* studies that provided evidence of antitumor activities [84, 85]. The transcriptional effector complex downstream of Notch has also been targeted using a different peptide-based approach. A hydrocarbon-stapled peptide antagonist of the Mastermind (MAML1) transcription factor inhibits Notch signaling in leukemic cells and in a murine T-ALL model [86]. In principle, these targeting strategies could be applied to other components of the Notch pathway, including ADAM10/17 proteases or Notch glycosylation enzymes, although an important consideration is the extent to which such agents might also disrupt other cellular processes that depend upon the same enzymes. Antibodies could also be used to target γ -secretase itself, an approach that has received little attention due to the availability of highly effective GSIs. However, human γ -secretase is heterogeneous, with at least six different complexes possible due to differential usage of either Presenilin-1 or -2 as well as Aph-1A_S, Aph-1A_L (two alternative Aph-1A splice variant-derived isoforms) or Aph-1B, and targeted inhibition of particular γ -secretase subtypes could potentially be beneficial in some therapeutic contexts. As proof of concept, an antibody against the extracellular domain of the γ -secretase component Nicastrin has been shown to inhibit γ -secretase activity by competing with substrate binding, and to interfere with proliferation of T-ALL cell lines and tumor growth in T-ALL mouse xenografts [87].

8. Conclusions and future prospects

As we near a full century of research on the Notch signaling pathway, the past decade has seen rapid advances in the development of therapeutic approaches to treat cancers and other maladies associated with abnormal Notch signaling. Most Notch-directed therapies involve the use of γ -secretase inhibitors, which were initially developed for Alzheimer's disease treatment, or antibodies that inhibit specific Notch receptors, ligands, or other pathway components. Promising results have also been obtained through less common approaches,

such as using Notch1 ectodomain expression to inhibit tumor growth and angiogenesis [88], inhibiting the ADAM metalloproteases that perform key activating cleavages of Notch [89], expressing dominant-negative fragments of Mastermind [90,91], and expressing Jag-1 and Dll-1 Fc fusion proteins to modulate Notch signaling [83]. Although all of these approaches show great potential for realistic therapeutic intervention of Notch signaling in the future, they also highlight the need for a better understanding of the role of each Notch paralogue in different diseases, the degree to which Notch activation is triggered by distinct ADAM enzymes and γ -secretase complexes, and the extent to which inhibition of one Notch paralogue can be compensated by upregulation or re-expression of other Notch paralogs. Of paramount importance will be biomarker studies on Notch-associated cancers to understand the other cellular events and signaling pathway interactions that contribute to tumor progression, thereby guiding the selection of the most effective therapeutic approach, which in many cases will involve GSI treatment or Notch immunotherapy in combination with other chemotherapeutic agents. Finally, it should be noted that Notch-targeting therapies are relevant not only for cancer, but also potentially for a host of developmental, vascular, cardiac, and other diseases associated with Notch pathway malfunction. As just one example, a mouse model for systemic sclerosis, characterized by organ fibrosis, vascular hyperreactivity, and immunological defects, responds favorably to GSI treatment through downmodulation of hyperactive Notch signaling [92].

Despite impressive recent progress in modulating Notch activity for therapeutic purposes, it is sobering to realize that as we approach the 100-year anniversary of Notch pathway research, aside from clinical trials, human patients are not yet routinely treated by deliberately targeting the Notch pathway. Nevertheless, just a few years after the 1913 isolation of the first *Drosophila Notch* mutant, the young scientist Merkel Henry Jacobs, who would go on to become an eminent figure in the field of membrane transport biology, called attention to the possibility of deliberately manipulating the growth and development of cells for practical purposes [93]:

The marvelous processes of development that occur in such an exact manner as to produce out of a speck of living matter one-eightieth of the weight of a postage stamp a human being that resembles one of his parents in minute details of appearance, bodily structure and disposition, or as to produce twins that are practically indistinguishable, are among the most wonderful of the various aspects of growth.

Surely it must require more than ordinary scientific boldness to make the attempt to control and utilize in new ways for human needs such forces as the ones we have been considering!

While it is difficult to imagine what is in store for the next century of research on Notch signaling, it is not inconceivable that we are on the cusp of a new era in which modulation of this pathway will become a widely accepted feature of medical treatment for human cancers and other diseases.

Acknowledgments

This work was supported by NIH R01 GM087650 from the National Institute of General Medical Sciences (NIGMS) and funding from the Thomas Jefferson University Department of Biochemistry. We apologize to our colleagues whose work we were unable to cite due to space limitations.

Abbreviations

GSI γ -secretase inhibitor

APP	amyloid precursor protein
T-ALL	T-cell acute lymphoblastic leukemia
NRR	negative regulatory region
ADAM	a disintegrin and metalloprotease
DII	Delta-like
NSCLC	non-small cell lung carcinoma

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Highlights

Dysregulated Notch signaling is associated with a wide variety of different human cancers

γ -secretase inhibitors block proteolytic activation of Notch and show promise for cancer therapy despite toxicity issues

Immunological targeting of specific Notch pathway components is an alternative approach to therapeutic modulation of Notch signaling

Figure 1. Alternative strategies to inhibit Notch signaling in cancer therapy

Notch receptors at the surface of the signal-receiving cell bind ligands presented by the signal-sending cell, resulting in structural changes in Notch that trigger its sequential cleavages by ADAM and γ -secretase proteases. Notch proteolysis leads to the release and nuclear translocation of the Notch intracellular domain (NICD), which directly regulates target gene transcription. Therapeutic strategies to modulate Notch pathway function include chemical and immunological targeting of Notch, its ligands, the ADAM and γ -secretase proteases, and the downstream transcriptional factor Mastermind, as indicated.

Figure 2. Structures of representative γ -secretase inhibitors

A) DFK167, a transition-state inhibitor [6]. B-D) DAPT [10], Compound E [9], and LY-450,139/semigacestat [12], three structurally related dipeptidic inhibitors. E) MK-0752, a sulfonamide inhibitor [12]. F) PF-03084014, a tetralin imidazole inhibitor [94].

Table 1
A representative survey of preclinical and clinical trials evaluating therapeutic targeting of Notch signaling in cancer

Clinical trials are listed with their www.ClinicalTrials.gov identifiers; references are given for reported preclinical and clinical trials.

Compound	Condition	Status	References
RO4929097	Breast cancer	Phase I clinical trials	
γ -secretase inhibitor (Roche)	Brain and CNS tumors	NCT01198535	
		NCT01218620	
	Colorectal cancer	NCT01217411	
	Melanoma	NCT01149356	
	Solid tumors	NCT01270438	
	T-cell leukemia	NCT01238133	
		NCT01088763	
		NCT01141569	
		NCT01208441	
		NCT01196416	
MRK-003	Breast cancer	Preclinical studies	[29, 71]
γ -secretase inhibitor (Merck & Co)	T-cell leukemia tumor xenografts		
MK-0752	Brain and CNS tumors	Phase I clinical trials	[95]
γ -secretase inhibition	γ -secretase inhibitor (Merck & Co)	NCT00756717	
	Breast cancer	NCT00803894	
	Neoplasms	NCT01295632	
	Pancreatic cancer	NCT01098344	
	T-cell leukemia	NCT00645333	
		NCT01243762	
		NCT00572182	
		NCT00106145	
		NCT00100152	
PF-03084014	Neoplasms	Phase I clinical trial	[26]
γ -secretase inhibitor (Pfizer)	Lymphoid leukemia	NCT00878189	
	Solid tumors		
	T-cell leukemia		
OMP-59R5	Solid tumors	Phase I clinical trial	
Anti-Notch mAb (OncoMed Pharmaceuticals)		NCT01277146	
Immunotherapy	OMP-21M18	Phase I clinical trials	[85]
	Anti-DLL4 mAb (OncoMed Pharmaceuticals)	NCT01189929	
	Small cell lung cancer	NCT01189942	
		NCT01189968	
	Solid tumors	NCT00744562	
NRR1	Anaplastic carcinoma	Preclinical study	[81]

	Compound	Condition	Status	References
	Anti-Notch1 mAb (Genentech & Exelixis)	Breast carcinoma		
		Colon carcinoma tumor xenografts		
	NRR1	T-cell leukemia	<i>In vitro</i> study	[82]
	Anti-Notch 1 mAb (Merck & Co)	T-ALL cell line		
	NRR2	Anaplastic carcinoma	Preclinical study	[81]
	Ana-Notch 1 mAb (Genentech & Exelixis)	Breast carcinoma		
		Colon carcinoma tumor xenografts		
	NRR3	HEK293T cell line	<i>In vitro</i> study	[80]
	Anti-Notch3 mAb (Genentech)			
	A5226A	T-cell leukemia	Preclinical study	[87]
	Anti-Nicastrin mAb	T-ALL tumor xenografts		
	DLL1-Fc & JAG1-Fc	Aberrant immune activation	<i>In vitro</i> study	[83]
	Anti-Delta-like 1 and Jagged 1 Fc chimeric mAbs			
Peptide	SAHM1	T-cell leukemia	Preclinical study	[86]
	MAM peptide antagonist (Aileron Therapeutics)	T-ALL tumor xenografts		