UCSF
UC San Francisco Previously Published Works

Title

Molecular Pathways: Context-Dependent Approaches to Notch Targeting as Cancer Therapy

Permalink

https://escholarship.org/uc/item/1tt5d15x

Journal

Clinical Cancer Research, 18(19)

ISSN

1078-0432

Authors

Egloff, Ann Marie
Grandis, Jennifer R

Publication Date

2012-10-01

DOI

10.1158/1078-0432.ccr-11-2258

Peer reviewed

Molecular Pathways: Context-dependent approaches to Notch targeting as cancer therapy

Ann Marie Egloff, PhD MPH[1,2,*] and **Jennifer R. Grandis, MD FACS**[1,3]

[1]Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15232

[2]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15232

[3]Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15232

Abstract

Recent high throughput genomic sequencing studies of solid tumors, including head and neck squamous cell carcinoma (SCC), ovarian cancer, lung adenocarcinoma, glioblastoma, breast cancer and lung SCC, have highlighted DNA mutation as a mechanism for aberrant Notch signaling. A primary challenge of targeting Notch for treatment of solid malignancies is determining whether Notch signaling is cancer-promoting or tumor-suppressing for a specific cancer. We compiled reported Notch receptor and ligand missense and nonsense mutations in order glean insights into aberrant Notch signaling.

Frequencies of coding mutations differed for the four Notch genes. 4.7% of tumors harbored NOTCH1 missense or nonsense mutations. NOTCH2 and NOTCH3 had similar overall mutation rates of 1.5% and 1.3%, respectively, while NOTCH4 mutations were rarer. Notch ligand genes were rarely mutated.

The combined mutation frequency and position spectra of the four Notch paralogs across the different cancers provide an opportunity to begin to illuminate the different contributions of each Notch paralog to each tumor type and to identify opportunities for therapeutic targeting. Notch signaling pathway activators and inhibitors are currently in early clinical development for treatment of solid malignancies. Defining the status and consequences of altered Notch signaling will be important for selection of appropriate treatment.

Background

The tumor microenvironment for solid malignancies involves a complex interplay of tumor cells, stromal matrix and support cells, blood vessel endothelial cells and immune cells. In order for solid tumors to progress and grow, an adequate blood supply is needed. The interplay between tumor cells and the endothelial cells of blood vessels will be vital to ensure the tumor is adequately supplied with nutrients. The stromal cells and matrix, originally thought to provide a relatively inert support for the process of tumorigenesis and tumor progression, have more recently been appreciated to be co-opted, active participants in these pathological processes. Notch signaling occurs at the interface of these microenvironment compartments (Figure 1).

*To whom correspondence should be addressed: Eye and Ear Institute, Suite 500, 200 Lothrop Street, Pittsburgh, PA 15213, TEL: 412-648-9545, egloffam@upmc.edu.

The authors declare no conflicts of interest

There are four Notch family receptors in humans, Notch1–4. Each of the four Notch receptors is initially produced as a single polypeptide that is cleaved by a furin-like convertase at site 1 (S1) while in transit through the Golgi apparatus to create non-covalently attached heterodimers. The extracellular amino-terminal portion of the Notch receptor contains a series of 29 to 36 epidermal growth factor-like (EGF) domains, specific subsets of which are involved in interactions with Notch ligands. A heterodimerization domain tethers the Notch extracellular domain to the carboxyl-terminal portion of the Notch receptor, which is comprised of an extracellular heterodimerization domain, a transmembrane domain and Notch intracellular domain (NICD). The canonical Notch ligands include Delta-like ligand (DLL) 1, 3 and 4 and Jagged1 (Jag1) and Jagged2 (Jag2). These ligands, similar to the Notch receptors, are single-pass transmembrane proteins with numerous extracellular EGF repeats.

Notch receptors are activated by a series of proteolytic events following productive ligand binding. Several excellent recent reviews provide detailed mechanisms of Notch including activation by non-canonical ligands (1) (2). Here, we highlight Notch domains and canonical Notch signaling pathway components currently recognized as most relevant for tumorigenesis (Figure 1). Ligand binding can result in Notch activation when the bound ligand is expressed on a cell adjacent to the Notch-expressing cell (*trans* interactions) or Notch inhibition when the bound ligand and Notch receptor are expressed on the same cell (*cis* interactions)(1). Distinct Notch EGF domains mediate the Notch-activating *trans* interactions and the inhibiting *cis* interactions with ligands (3). In addition to the EGF repeats within the extracellular domain, three Lin-12/Notch repeats (LNRs) protect against activating cleavage at site 2 (S2), which is approximately 12 amino acids before the transmembrane domain, until it is appropriately exposed following ligand binding. *Trans* interactions between ligand and Notch provide the TACE/ADAM (a disintegrin and metaloprotease) metalloproteinases access to the Notch S2, permitting cleavage at S2 and the removal of the ectodomain (1). The loss of the ectodomain results in a membrane-tethered intermediate that is a substrate for γ-Secretase, a multicomponent intramembrane protease (1).

Cleavage of Notch receptors by γ-Secretase is required for the release from the membrane of the NICD, which then translocates to the nucleus. The NICD regulates transcription of target genes through interactions with transcriptional machinery partners, including the CBF-1, Su(H), Lag-1 (CSL) DNA-binding transcription factor via the Notch ankyrin repeats and the Mastermind transcriptional coactivators (MAML1–3) via the Notch RAM motif (4). Notch 1 and Notch 2 each contain a transcriptional activation domain (TAD) following the anykrin repeats within the NICD, but the TAD is lacking for Notch 3 and Notch 4. Located near the carboxyl-terminus of the NICD is the PEST domain, which can be ubiquitinated by the FBXW7-containing E3 ubiquitin ligase complex targeting the NICD for destruction. The specific unique biological functions of the four different Notch receptors and how these activities differ by ligand are not well understood.

Signaling events in Notch ligand-expressing cells are likely important, though as yet poorly understood, contributors to tumorigenesis. Similar to Notch receptors, Notch ligands undergo sequential proteolytic cleavage following receptor binding and have been reported to activate transcription (5). An intact PDZ- (PSD-95/DLG/ZO-1)-ligand domain, which is present at the carboxyl terminus of Notch ligands, was reported to be essential for Jag1-conferred transformation of immortalized rat kidney cells *in vitro* (6). The DLL4 Notch ligand is highly expressed at the tips and stalks of sprouting vessel endothelial cells and is an important regulator of angiogenesis (7).

The Notch signaling pathway has long been appreciated as functioning in developmental processes and regulating the self-renewal of tissues. More recently, the role of Notch signaling in cancers has been reported with Notch signaling having both oncogenic and tumor suppressive roles, depending on the cellular context. Activating mutations in *NOTCH1* were indentified in T cell acute lymphoblastic leukemia and chronic lymphoblastic leukemia (8–10), implicating *NOTCH1* as an oncogene for these hematopoietic cancers. In the absence of reported *NOTCH* mutations in solid malignancies prior to 2011, there was already an appreciation that the contribution of Notch signaling to tumor development was largely context dependent, with Notch contributing to tumor development in some instances while suppressing tumorigenesis in other contexts. There have been a number of excellent recent reviews discussing these nuances of Notch signaling (11–14). Our goal for this article is to highlight available *NOTCH* mutation and published functional/correlative data for solid malignancies in order to begin to more fully appreciate the different roles of Notch signaling in cancer and opportunities for therapeutic targeting.

NOTCH receptor mutations in solid malignancies

A number of groups have investigated Notch receptor and ligand mutation status in various tumor types either through whole exome sequence analysis (head and neck squamous cell carcinoma (HNSCC) (15–16), ovarian carcinoma (17), colorectal and breast cancers (18)) or by sequence analysis of entire candidate gene exons including Notch receptors and ligands (breast cancer (19), glioblastoma (20), lung adenocarcinoma (21), and lung squamous cell carcinoma (lung SCC)(22)). Review of these data for nonsilent protein-coding mutations indicated that NOTCH ligands were rarely mutated: *JAG1* was mutated in 1 of 91 sequenced glioblastomas and *JAG2* was mutated in 2 of 188 lung adenocarcinomas (20) (21). Notch receptors were mutated in several tumor types, and the frequency of mutation of specific Notch receptors and the location of the mutations within each Notch receptor varied markedly by tumor type (Figure 2). We confined our analysis to nonsilent protein coding alterations whose protein product could be predicted and did not evaluate splice site mutations. Mutations that were reported to be tested but were not confirmed in validation studies were excluded.

Specific functional consequences of mutated Notch receptors and ligands are largely not yet experimentally defined, but the clustering of mutations in known functional elements invites speculation regarding whether the specific Notch receptor likely functions as a tumor suppressor or oncogene in a specific cancer type (Figure 2).

More *NOTCH1* gene mutations were observed than mutations in the other *NOTCH* receptor genes. This was in part, but not entirely, due to the greater number of tumors with *NOTCH1* sequencing data. For HNSCC, lung SCC and breast, *NOTCH1* mutations were relatively frequent, with 5–15% of tumors harboring protein coding changes. Many of these missense mutations occurred at or near identified important domains such as the ligand-binding domain (EGF repeats 11 and 12) or the ankyrin domains (Figure 2). Nonsense mutations observed in HNSCC would be predicted to result in truncated Notch1 proteins lacking important domains for transcription activation. The data suggest that Notch1 may be acting as a tumor suppressor in these tumor types. Though it is formally possible that secreted truncated Notch1 proteins may be gain of function mutants, there are no data to support this notion.

Despite the prevalence of *NOTCH1* mutations in HNSCC, the published data are sparse and conflicting regarding the function of Notch1 in these tumors. Notch1 protein levels have been found to be increased in HNSCC compared to adjacent mucosal tissues (23), and HNSCC tumors expressing higher levels of Notch1 protein were associated with reduced patient survival (24). However, in separate studies the expression of an activated form of

Notch1, the NICD, was reported to result in enhanced or greatly diminished HNSCC cell line *in vivo* tumorigenicity (24–25).

The increased frequency of *NOTCH1* mutations in lung SCC compared to lung adenocarcinoma and the close proximity of two of the three lung SCC mutations to the ligand-binding domain invites speculation that Notch1 is more likely to function as a tumor suppressor in lung SCC than adenocarcinoma. These lung SCC mutations had a distribution that was similar to cutaneous SCC mutations identified in the same published study, a subset of which were characterized to be loss of function mutations by a cell-based transcription reporter assay (22). Possible differences in Notch1 function in lung adenocarcinoma versus lung SCC are highlighted by a single report that high tumor Notch1 protein levels were associated with significantly reduced survival for lung adenocarcinoma patients (P=0.004, n=111) but not for lung SCC patients (P=0.23, n=188)(26). However, reported functions of Notch1 in lung adenocarcinoma are conflicting (27–28).

Though *NOTCH1* mutations in breast cancer include a missense mutation within the ligand-binding domain and a spectrum that does not differ appreciably from those observed in HNSCC, Notch1 studies in breast cancers have generally reported functional or tumor molecular data implicating Notch1 as an oncogene in these cancers (29–30). *NOTCH1* mutations occurring in glioblastoma were infrequent and did not map to known functional domains. The functional/correlative data for Notch1 in glioma from two independent reports consistently implicate *NOTCH1* as an oncogene (31–32). One of the observed *NOTCH1* mutations in ovarian carcinoma, P2417A, has also been observed in acute T-lymphoblastic leukemia and maps within PEST domain (33).

The Notch3 ligand-binding domains are presented in Figure 2 as recently defined by functional studies employing a competitive peptide library screen to include EGF repeats 7–10 and 21–22 (34). Mutations observed in HNSCC, ovarian cancer and lung adenocarcinoma occurred in about 1% of these tumors and generally resided within or near recognized functional domains, suggesting these mutations may be loss of function mutations. *NOTCH3* sequence data were not yet available for lung SCC.

The implications of the spectrum of mutations for *NOTCH2* and *NOTCH4* receptors were more veiled. The ligand-binding domain of Notch2 has been defined based on extrapolation from Notch1. No *NOTCH2* mutations were observed in the putative ligand-binding domain. The Notch4 ligand-binding domain has not been defined. Nonsense mutations observed in HNSCC and lung SCC suggests that Notch2 may function as a tumor suppressor in these cancer types. The observation of mutations in *FBXW7* in approximately 5% of HNSCC in two independent studies and 1% of ovarian cancers is a reminder that while considering mutation spectra, there exist other important aspects of Notch regulation that cannot be gleaned from these data (15–17). Of course, it is possible that the functional consequences of the observed mutations will differ from predictions. More likely, there exist subgroups within each of these tumor types with differential dependence upon gain or loss of the different Notch receptor activities.

A caveat of comparing gene mutation profiles where the number of tumors varies appreciably by cancer site is bias due to sample selection. Relatively small sample sizes may not reflect the study population at large and therefore subsets from each study may not reflect general characteristics. Admittedly, gene mutation spectra are but one aspect of a complex story. Projects such as The Cancer Genome Atlas (TCGA; (35)) will be especially informative for defining the roles of the different Notch receptors in different cancers. By characterizing tumor mutations, copy number alterations, gene expression profiles and gene methylation patterns in a large number of tumors, it is hoped that clinically relevant subtypes

of organ-specific cancers will be identified through the TCGA and other efforts and eventually effective therapies as well.

Clinical-Translational Advances

Notch pathway-targeting agents in clinical development for solid malignancies

The appropriate Notch pathway-directed therapeutic is anticipated to differ depending upon whether the tumor harbors a Notch alteration resulting in gain or loss of function. An approach to generally inactivate Notch signaling via inhibition of the γ-Secretase is currently being evaluated as a possible anti-cancer strategy for tumors with acquired Notch gain of function. γ-Secretase has been implicated in Alzheimer's disease as one of the enzyme complexes responsible for cleaving amyloid precursor protein (APP) into amyloid beta, a primary component of amyloid plaques. Because of these pathogenic implications, γ-Secretase inhibitors are in clinical development for several diseases including solid malignancies (Table 1).

The finding that a γ-Secretase inhibitor (GSI) resulted in increased incidence of skin cancers with no improvement in Alzheimer's disease symptoms lead to the halt of a large phase III clinical trial (36). This skin toxicity has been largely attributed to Notch inhibition, and as a result, Notch-sparing GSIs are being developed for treatment of Alzheimer's disease (37–39). The accumulating preclinical and clinical data indicating that Notch is oncogenic in several tumor types has lead to the recent clinical development of GSIs that are not Notch-sparing for treatment of solid tumors.

There are several GSIs that are currently in Phase I or Phase I/II trials for various malignancies (Table 1). Reports from these early phase trials indicate that the inclusion of biomarkers including tumor levels of Notch proteins, NICD, Notch transcriptional target genes such as HES1 and HEY1 and blood biomarker evaluations were planned for several of these studies (40–42). In general, toxicities were manageable. An intermittent dosing schedule appeared to ameliorate gastrointestinal toxicities compared to a continuous dosing regimen (40, 43). The majority of tumors were positive for Notch1 in trials reporting results (40–41). Reported response rates were modest (40–41) and low baseline levels of IL6 and IL8 were associated with clinical benefit (42).

The finding that the Notch ligand DLL4 is an important regulator of angiogenesis lead to the characterization and development of DLL4-directed therapeutics as a means for interfering with tumor vascularization (Table 1). No results have been reported for the anti-DLL4 antibody therapeutic, MEDI0639. However the finding that chronic treatment with anti-DLL4 resulted in vascular neoplasms in mice indicates that treatment management will require extra care (44).

Now that Notch1 has been identified as a tumor suppressor, at least in some tissues, administering systemic therapeutics that inhibit general Notch signaling is anticipated to require careful management to reduce additional cancer risks. This would be especially true for patients with early stage disease where the risks of a secondary cancer may outweigh the possible benefits.

Notch-activating agents in clinical development for solid malignancies

Epigenetic silencing of Notch and other tumor suppressor genes is a prominent mechanism for tumor development. Application of agents that interfere with epigenetic silencing of Notch has been reported to reduce tumor-like phenotypes in several preclinical models including neuroendocrine tumors, medullary thyroid cancers and others (45–48). Histone deacetylase (HDAC) inhibitors have had some success with NOTCH re-expression in

cancers where NOTCH expression was lost by this mechanism. There are two HDAC inhibitors currently in clinical development (Table 1), and efforts to identify Notch pathway compounds are ongoing (48). Results from a phase II study of valproic acid (VPA) involving 8 patients with neuroendocrine tumors indicated that Notch1 expression was absent from tumors prior to treatment and increased significantly following VPA treatment. Four of these patients had stable disease as best response (49).

Tumors harboring more than one predicted *NOTCH* inactivating mutation are infrequent (15–16). Formal possibilities are that the wildtype allele is present and expressed appropriately in tumors with mutated *NOTCH* and, therefore, loss of function *NOTCH* mutations would be described as haploinsufficient. Alternatively, the mutations predicted to be loss of function mutations are in fact gain of function mutations acting through an as yet unknown mechanism. An HDAC inhibitor would not be a rational solution in either case. However, if the wildtype allele is present but transcriptionally repressed, as has been observed for BRCA1 mutations in breast cancers, an HDAC inhibitor may be a viable therapeutic for tumors harboring a *NOTCH* inactivating mutation. As we learn more about the context and functional consequences of specific *NOTCH* mutations, we will be better poised to identify appropriate therapeutics for the increasing number of solid tumors with identified *NOTCH* mutations.

A better understanding of the specific Notch signaling pathway alterations in the context of each cancer is warranted. We posit that the nuances and ambiguities of the roles of Notch receptor signaling in cancers may be defined at least in part by the specific Notch pathway mutation, Notch receptor and ligand expression alteration and concomitant gene expression profile. Ongoing efforts such as those by the TCGA will greatly facilitate the development of the contextual understanding of Notch and other tumor-specific alterations.

References

1. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. Cell. 2009; 137:216–233. [PubMed: 19379690]

2. Fortini ME. Notch signaling: the core pathway and its posttranslational regulation. Dev Cell. 2009; 16:633–647. [PubMed: 19460341]

3. de Celis JF, Bray SJ. The Abruptex domain of Notch regulates negative interactions between Notch, its ligands and Fringe. Development. 2000; 127:1291–1302. [PubMed: 10683181]

4. Kovall RA. More complicated than it looks: assembly of Notch pathway transcription complexes. Oncogene. 2008; 27:5099–5109. [PubMed: 18758478]

5. LaVoie MJ, Selkoe DJ. The Notch ligands, Jagged and Delta, are sequentially processed by alpha-secretase and presenilin/gamma-secretase and release signaling fragments. J Biol Chem. 2003; 278:34427–34437. [PubMed: 12826675]

6. Ascano JM, Beverly LJ, Capobianco AJ. The C-terminal PDZ-ligand of JAGGED1 is essential for cellular transformation. J Biol Chem. 2003; 278:8771–8779. [PubMed: 12496248]

7. Hellstrom M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, et al. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. Nature. 2007; 445:776–780. [PubMed: 17259973]

8. Ellisen LW, Bird J, West DC, Soreng AL, Reynolds TC, Smith SD, et al. TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell. 1991; 66:649–661. [PubMed: 1831692]

9. Weng AP, Ferrando AA, Lee W, Morris JPt, Silverman LB, Sanchez-Irizarry C, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science. 2004; 306:269–271. [PubMed: 15472075]

10. Puente XS, Pinyol M, Quesada V, Conde L, Ordonez GR, Villamor N, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature. 2011; 475:101–105. [PubMed: 21642962]

11. Ranganathan P, Weaver KL, Capobianco AJ. Notch signalling in solid tumours: a little bit of everything but not all the time. Nat Rev Cancer. 2011; 11:338–351. [PubMed: 21508972]

12. Lobry C, Oh P, Aifantis I. Oncogenic and tumor suppressor functions of Notch in cancer: it's NOTCH what you think. J Exp Med. 2011; 208:1931–1935. [PubMed: 21948802]

13. Radtke F, Raj K. The role of Notch in tumorigenesis: oncogene or tumour suppressor? Nat Rev Cancer. 2003; 3:756–767. [PubMed: 14570040]

14. Roy M, Pear WS, Aster JC. The multifaceted role of Notch in cancer. Curr Opin Genet Dev. 2007; 17:52–59. [PubMed: 17178457]

15. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science (New York, N Y). 2011; 333:1157–1160.

16. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science (New York, N Y). 2011; 333:1154–1157.

17. Integrated genomic analyses of ovarian carcinoma. Nature. 2011; 474:609–615. [PubMed: 21720365]

18. Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, et al. The genomic landscapes of human breast and colorectal cancers. Science. 2007; 318:1108–1113. [PubMed: 17932254]

19. Jiao X, Wood LD, Lindman M, Jones S, Buckhaults P, Polyak K, et al. Somatic mutations in the Notch, NF-KB, PIK3CA, and Hedgehog pathways in human breast cancers. Genes Chromosomes Cancer. 2012; 51:480–489. [PubMed: 22302350]

20. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008; 321:1807–1812. [PubMed: 18772396]

21. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008; 455:1069–1075. [PubMed: 18948947]

22. Wang NJ, Sanborn Z, Arnett KL, Bayston LJ, Liao W, Proby CM, et al. Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. Proc Natl Acad Sci U S A. 2011; 108:17761–17766. [PubMed: 22006338]

23. Zhang TH, Liu HC, Zhu LJ, Chu M, Liang YJ, Liang LZ, et al. Activation of Notch signaling in human tongue carcinoma. J Oral Pathol Med. 2011; 40:37–45. [PubMed: 20819128]

24. Lin JT, Chen MK, Yeh KT, Chang CS, Chang TH, Lin CY, et al. Association of high levels of Jagged-1 and Notch-1 expression with poor prognosis in head and neck cancer. Ann Surg Oncol. 2010; 17:2976–2983. [PubMed: 20517681]

25. Duan L, Yao J, Wu X, Fan M. Growth suppression induced by Notch1 activation involves Wnt-beta-catenin down-regulation in human tongue carcinoma cells. Biol Cell. 2006; 98:479–490. [PubMed: 16608439]

26. Donnem T, Andersen S, Al-Shibli K, Al-Saad S, Busund LT, Bremnes RM. Prognostic impact of Notch ligands and receptors in nonsmall cell lung cancer: coexpression of Notch-1 and vascular endothelial growth factor-A predicts poor survival. Cancer. 2010; 116:5676–5685. [PubMed: 20737536]

27. Allen TD, Rodriguez EM, Jones KD, Bishop JM. Activated Notch1 induces lung adenomas in mice and cooperates with Myc in the generation of lung adenocarcinoma. Cancer Res. 2011; 71:6010–6018. [PubMed: 21803744]

28. Zheng Q, Qin H, Zhang H, Li J, Hou L, Wang H, et al. Notch signaling inhibits growth of the human lung adenocarcinoma cell line A549. Oncol Rep. 2007; 17:847–852. [PubMed: 17342326]

29. Reedijk M, Odorcic S, Chang L, Zhang H, Miller N, McCready DR, et al. High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. Cancer Res. 2005; 65:8530–8537. [PubMed: 16166334]

30. Lee CW, Simin K, Liu Q, Plescia J, Guha M, Khan A, et al. A functional Notch-survivin gene signature in basal breast cancer. Breast Cancer Res. 2008; 10:R97. [PubMed: 19025652]

31. Chen J, Kesari S, Rooney C, Strack PR, Shen H, Wu L, et al. Inhibition of notch signaling blocks growth of glioblastoma cell lines and tumor neurospheres. Genes Cancer. 2010; 1:822–835. [PubMed: 21127729]

32. Wang J, Wakeman TP, Lathia JD, Hjelmeland AB, Wang XF, White RR, et al. Notch promotes radioresistance of glioma stem cells. Stem Cells. 2010; 28:17–28. [PubMed: 19921751]

33. Baldus CD, Thibaut J, Goekbuget N, Stroux A, Schlee C, Mossner M, et al. Prognostic implications of NOTCH1 and FBXW7 mutations in adult acute T-lymphoblastic leukemia. Haematologica. 2009; 94:1383–1390. [PubMed: 19794083]

34. Lin L, Mernaugh R, Yi F, Blum D, Carbone DP, Dang TP. Targeting specific regions of the Notch3 ligand-binding domain induces apoptosis and inhibits tumor growth in lung cancer. Cancer Res. 2010; 70:632–638. [PubMed: 20068176]

35. The Cancer Genome Atlas. [Available from: http://cancergenome.nih.gov/

36. [cited 2010 August 17, 2010] Lilly Halts Development of Semagacestat for Alzheimer's Disease Based on Preliminary Results of Phase III Clinical Trial. 2010. Available from: http://files.shareholder.com/downloads/LLY/1804235759x0x395879/54b1f68f-c7b8-4c04-87d1-8c609c21f6f7/LLY_News_2010_8_17_Product.pdf

37. Kreft A, Harrison B, Aschmies S, Atchison K, Casebier D, Cole DC, et al. Discovery of a novel series of Notch-sparing gamma-secretase inhibitors. Bioorg Med Chem Lett. 2008; 18:4232–4236. [PubMed: 18556202]

38. Gu H, Deng Y, Wang J, Aubry AF, Arnold ME. Development and validation of sensitive and selective LC-MS/MS methods for the determination of BMS-708163, a gamma-secretase inhibitor, in plasma and cerebrospinal fluid using deprotonated or formate adduct ions as precursor ions. J Chromatogr B Analyt Technol Biomed Life Sci. 2010; 878:2319–2326.

39. Tong G, Wang JS, Sverdlov O, Huang SP, Slemmon R, Croop R, et al. Multicenter, Randomized, Double-Blind, Placebo-Controlled, Single-Ascending Dose Study of the Oral gamma-Secretase Inhibitor BMS-708163 (Avagacestat): Tolerability Profile, Pharmacokinetic Parameters, and Pharmacodynamic Markers. Clin Ther. 2012; 34:654–667. [PubMed: 22381714]

40. Fouladi M, Stewart CF, Olson J, Wagner LM, Onar-Thomas A, Kocak M, et al. Phase I trial of MK-0752 in children with refractory CNS malignancies: a pediatric brain tumor consortium study. J Clin Oncol. 2011; 29:3529–3534. [PubMed: 21825264]

41. Strosberg JR, Yeatman T, Weber J, Coppola D, Schell MJ, Han G, et al. A phase II study of RO4929097 in metastatic colorectal cancer. Eur J Cancer. 2012; 48:997–1003. [PubMed: 22445247]

42. He W, Luistro L, Carvajal D, Smith M, Nevins T, Yin X, et al. High tumor levels of IL6 and IL8 abrogate preclinical efficacy of the gamma-secretase inhibitor, RO4929097. Mol Oncol. 2011; 5:292–301. [PubMed: 21315665]

43. Krop IE, Kosh M, Fearen I, Savoie J, Dallob A, MAtthews C, et al. Phase I pharmacokinetic (PK) and pharmacodynamic (PD) trial of the novel oral Notch inhibitor MK-0752 in patients (pts) with advanced breast cancer (BC) and other solid tumors. Journal of Clinical Oncology, 2006 ASCO Annual Meeting Proceedings Part I. 2006; 24 Abstract10574.

44. Yan M, Callahan CA, Beyer JC, Allamneni KP, Zhang G, Ridgway JB, et al. Chronic DLL4 blockade induces vascular neoplasms. Nature. 2010; 463:E6–E7. [PubMed: 20147986]

45. Truong M, Cook MR, Pinchot SN, Kunnimalaiyaan M, Chen H. Resveratrol induces Notch2-mediated apoptosis and suppression of neuroendocrine markers in medullary thyroid cancer. Ann Surg Oncol. 2011; 18:1506–1511. [PubMed: 21184191]

46. Adler JT, Hottinger DG, Kunnimalaiyaan M, Chen H. Histone deacetylase inhibitors upregulate Notch-1 and inhibit growth in pheochromocytoma cells. Surgery. 2008; 144:956–961. discussion 61-2. [PubMed: 19041003]

47. Greenblatt DY, Vaccaro AM, Jaskula-Sztul R, Ning L, Haymart M, Kunnimalaiyaan M, et al. Valproic acid activates notch-1 signaling and regulates the neuroendocrine phenotype in carcinoid cancer cells. Oncologist. 2007; 12:942–951. [PubMed: 17766653]

48. Pinchot SN, Jaskula-Sztul R, Ning L, Peters NR, Cook MR, Kunnimalaiyaan M, et al. Identification and validation of Notch pathway activating compounds through a novel high-throughput screening method. Cancer. 2011; 117:1386–1398. [PubMed: 21425138]

49. Mohammed TA, Holen KD, Jaskula-Sztul R, Mulkerin D, Lubner SJ, Schelman WR, et al. A pilot phase II study of valproic acid for treatment of low-grade neuroendocrine carcinoma. Oncologist. 2011; 16:835–843. [PubMed: 21632454]
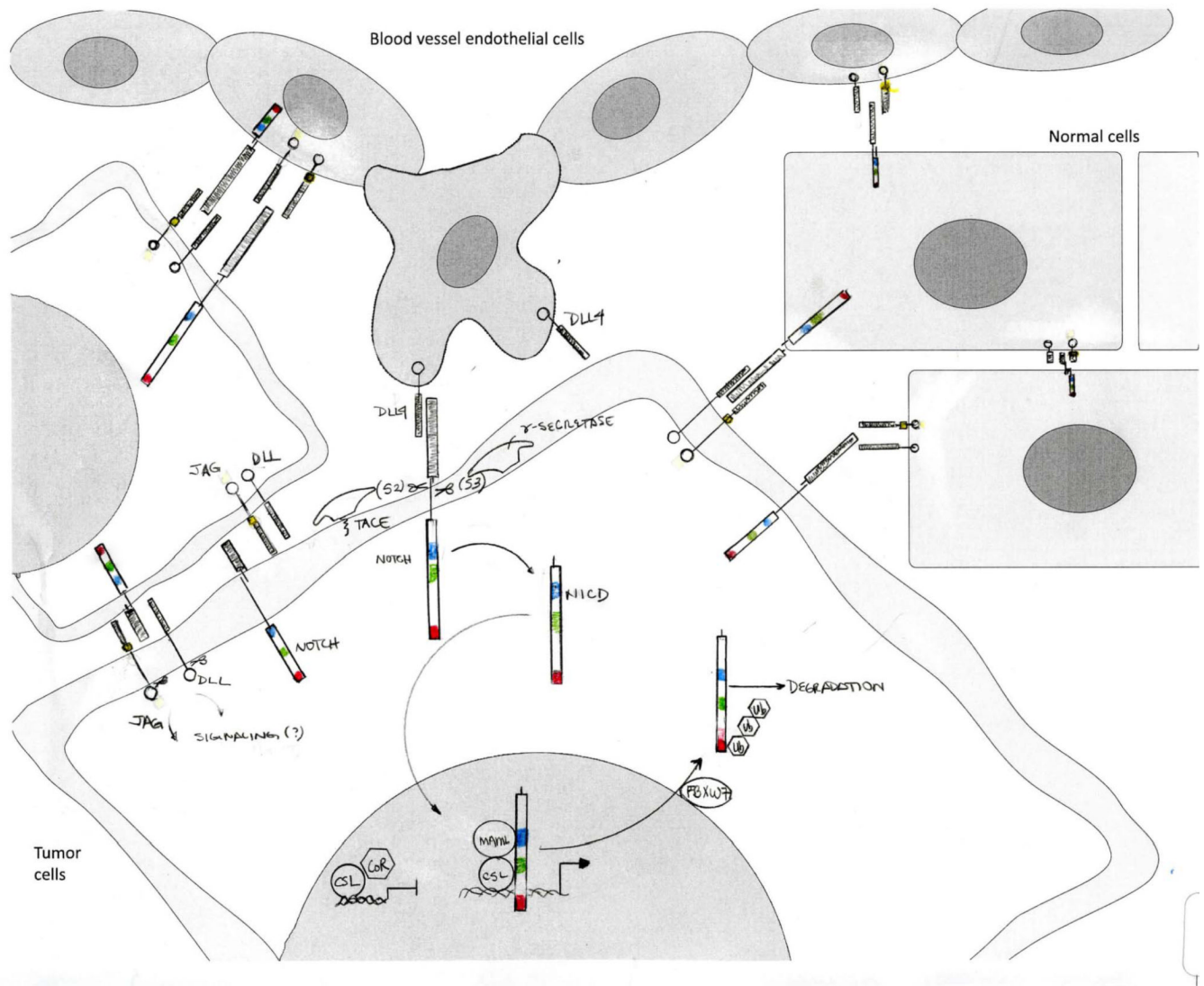
Figure 1. Notch signaling within the tumor microenvironment is multidirectional
Notch receptors and ligands are expressed in tumor cells, normal cells and endothelial vessel
cells, and productive interactions between Notch receptors and ligands take place at these
interfaces. The Notch ligand DLL4 is expressed at tip cells of budding vasculature while
Notch receptors and other ligands are largely excluded. Expression of specific Notch
receptors and ligands and their altered levels in each cellular compartment will vary
depending upon cancer type and milieu of accompanying alterations associated with the
pathogenic state. Signal-initiating interactions between EGF domains of Notch receptors and
EGF domains of either the DLL or Jag ligands lead to cleavage of Notch first by ADAM/
TACE proteases followed by γ-Secretase. This two-step cleavage of Notch liberates the
NICD containing the RAM domain (blue), ankyrin domains (green) and PEST domain (red).
Liberated from the membrane tether, NCID can move into the nucleus, interact with
transcriptional regulators including the DNA-binding protein CSL, displace transcriptional
co-repressors (CoR), and recruit transcriptional activators (MAML) to activate transcription.
Levels of Notch proteins are regulated in part by ubiquitination and degradation processes
involving FBXW7. Activation of Notch signaling may occur in any or all of the three
cellular compartments. DLL and Jag ligands, which harbor putative carboxyl-terminal PDZ

ligand domains (open circles), are also cleaved following activation and may initiate signaling events, some via the interaction with PDZ domain-containing proteins. Jag ligands each have a cysteine-rich domain (yellow) between the EGF repeat and the transmembrane domain. This cysteine-rich domain, whose function is not known, is absent in the DLL ligands.
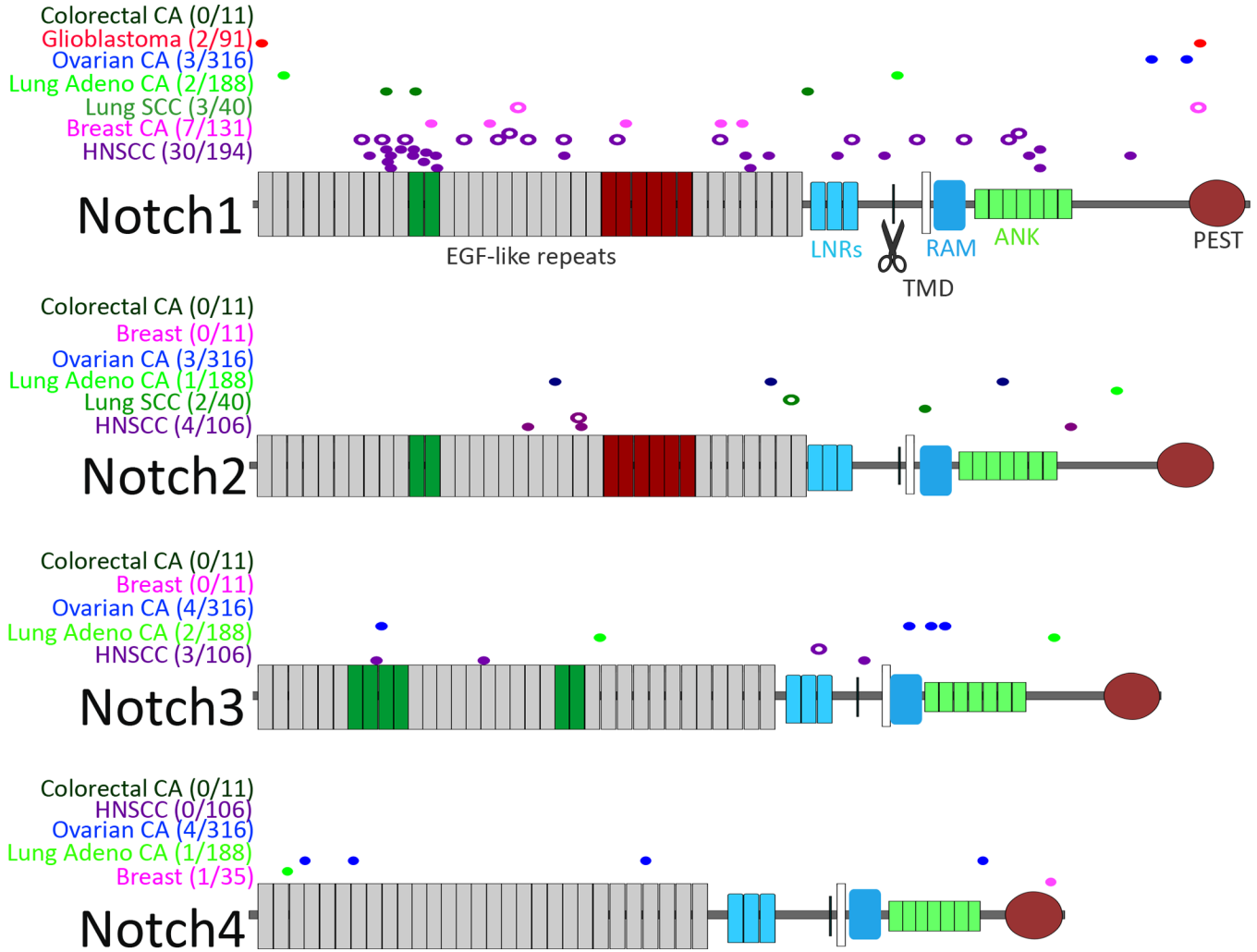
Figure 2. *NOTCH* **coding mutation spectra differ by paralog and cancer type**
Schematics of Notch1–4 with defined domains including the EGF-like repeats, the Lin12/Notch repeats (LNRs), furin-like convertase protease cleavage site (S1) (scissors), RAM domain, ankyrin repeats (ANK) and PEST destruction domain. EGF repeats implicated in ligand binding leading to Notch activation are indicated in green. EGF repeats mediating inhibitory effects of ligands binding in cis are indicated in red. Each cancer type is color coded and number of tumors harboring a NOTCH gene mutation and number of tumors sequenced are provided as the numerator and denominator, respectively in parentheses. Few tumors had more than 1 mutation. Closed circles are missense or single amino acid deletion mutations; open circles are nonsense mutations.

Table 1

Notch Pathway-Targeted Agents in Clinical Development for Solid Malignancies

Pathway Alteration	Drug	Mechanism of Action	Company	Phase	Cancer
Notch pathway inhibitors	MK0752	γ-Secretase inhibitor	Merck	I/II	Breast/ Pancreatic
	MK0752	γ-Secretase inhibitor	Merck	I	Pediatric CNS
	RO4929097; R4733	γ-Secretase inhibitor	Roche	I/II	Breast/Prostate/NSCLC/ Colorectal/Melanoma/ Kidney/Malignant Glioma/ Glioblastoma/ Pancreatic/ Sarcoma/CNS
	RO4929097; R4733	γ-Secretase inhibitor	Roche	I	Breast/ NSCLC/ Colorectal/ Glioma/ Pancreatic/ Adv. Solid tumors
	BMS-906024	γ-Secretase inhibitor	BMS	I	Adv. solid tumors
	PF-03084014	γ-Secretase inhibitor	Pfiser	I	Adv. solid tumors
	MEDI0639	Anti-DLL4 antibody	MedImmune LLC	I	Adv. solid tumors
Notch pathway activators	LBH589; Panobinostat	Histone Deacetylase inhibitor	Novartis	II	Thyroid/ Neuroendocrine
	Valproic Acid	Histone Deacetylase inhibitor	NA	II	Thyroid/Prostate/ Pancreatic/Cervical/ Breast/ Glioma/SCLC/
	Valproic Acid	HistoneM Deacetylase inhibitor	NA	I/II	NSCLC/Melanoma/ Sarcoma/Malignanant Mesothelioma/Ovarian
	Valproic Acid	Histone Deacetylase inhibitor	NA	I	Breast/NSCLC/Brain and CNS/Sarcoma/ NPC/Adv. Solid tumors

Central nervous system (CNS), non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), nasopharyngeal cancer (NPC), advanced solid malignancies (Adv. solid tumors)
Clinical trials were compiled from ClinicalTrials.gov