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The Double-Edged Sword of Notch Signaling in Cancer

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Abstract

Recent deep sequencing of cancer genomes has produced an explosion of new data implicating Notch signaling in several human cancers. Unlike most other pathways, these data indicate that Notch signaling can be either oncogenic or tumor suppressive, depending on the cellular context. In some instances, these relationships were predicted from mouse models or presaged by developmental roles for Notch, but in other cases were unanticipated. This review discusses the pathogenic and translational significance of these new findings.

Keywords

Notch signaling; tumor suppressor; oncogene

1. Introduction

Notch receptors control many aspects of development and homeostasis in multicellular animals via a signal transduction pathway that relies on regulated intramembranous proteolysis (for recent review, see [1]). Mammals have four Notch receptors, Notch1–4 (Fig. 1). The extracellular domain of these proteins consists of variable numbers of epidermal growth factor(EGF)-like repeats that participate in ligand-binding, three Lin12/Notch repeats (LNRs), and a juxtamembrane heterodimerization domain. During maturation, Notch receptors are cleaved within the heterodimerization domain by furin-like proteases, producing two subunits that associate non-covalently through contacts in the heterodimerization domain and the LNRs, which together constitute a negative regulatory region (NRR) that is responsible for preventing ligand-independent receptor activation. The intracellular portions of Notch receptors include RAM and ankyrin repeat domains that are involved in protein:protein interactions and a C-terminal PEST degron domain.

Notch signaling is normally initiated by ligands expressed on neighboring cells that belong to the Delta-Serrate-Lag2 (DSL) family, which are also transmembrane proteins (Fig. 1).

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Ligand binding first triggers cleavage by ADAM-10 or ADAM-17 metalloproteases at a site just external to the transmembrane domain, creating a short-lived membrane-bound intermediate lacking most of the Notch ectodomain that is a substrate for γ-secretase, a multisubunit intramembranous protease. Cleavage by g-secretase releases the intracellular domain of Notch (ICN), which translocates to the nucleus and forms a short-lived transcription activation complex with the DNA-binding factor RBPJ (also known as CSL) and coactivators of the MAML family. Rapid turnover of this complex is normally ensured in part by the C-terminal PEST degron in ICN.

Although some studies point to transcription-independent crosstalk between Notch and other signaling pathways such as Wnt and PI3K/Akt, genetic studies suggest that most Notch effects are mediated through transactivation of target genes by the RBPJ/ICN/MAML transcription complex. The outcome of signaling through this “canonical” pathway is strongly influenced by dose and cellular context; indeed, the lack of enzymatic amplification and putative transcription coupled degradation of ICN means that one activated Notch receptor probably transactivates at most one target gene prior to degradation, allowing for precise regulation of signal strength and duration.

Depending on the cellular context, Notch signaling can be an arbiter of survival versus death; proliferation versus growth arrest; or differentiation versus “stemness”. Given these varied effects, it is not surprising that widely divergent context-dependent roles for Notch have emerged in cancer. It is well established that Notch1 acts as an oncoprotein in T-cell acute lymphoblastic leukemia/lymphoma (T-ALL), an aggressive tumor that occurs mainly in children and adolescents. Recent application of deep sequencing approaches to hundreds of human cancer genomes and transcriptomes has detected somatic mutations in Notch receptor genes in an increasingly wide spectrum of tumors, expanding the breadth of Notch’s roles in cancer. Mouse modeling foretold some of these discoveries, but others were unexpected. This review mainly focuses on these new findings, which clearly document Notch’s ability to function as either an oncoprotein or a tumor suppressor depending on cellular context.

2. Notch as an oncoprotein: Notch1 in T-ALL as a paradigm

Before delving into recent discoveries (summarized in Table 1 and Table 2), brief review of oncogenic Notch mutations in T-ALL is in order as a point of reference (for detailed review, see [2]). To date somatic gain-of-function mutations in human T-ALL are confined to *NOTCH1* and fall into two general classes. The most common class consists of point substitutions or small in-frame deletions, insertions or duplications involving the Notch1 NRR or transmembrane domain that allow ligand-independent proteolysis and activation. The second class consists of nonsense or frameshift mutations that result in the deletion of the C-terminal PEST degron domain. Some T-ALLs possess mutations of both classes in *cis*, an alignment that produces synergistic increases in signaling. Rarely in human T-ALL the *NOTCH1* locus is broken by (7;9) translocations that fuse the 3’ end of *NOTCH1* to *TCRB* promoter/enhancer elements. These rearranged *NOTCH1* alleles express truncated transcripts encoding polypeptides that lack the NRR entirely. Similarly, in murine T-ALLs *notch1* is commonly disrupted by RAG-mediated deletions that remove the 5’ end of the gene and activate a cryptic internal promoter that drives the expression of mRNAs encoding truncated polypeptides lacking the NRR. Thus, in T-ALL there is strong selection for somatic mutations that disrupt the Notch1 NRR and permit ligand-independent receptor activation. In the case of T-ALL, ligand-independent signaling may promote the spread of the tumor beyond the confines of the ligand-rich thymic microenvironment.

Another theme emerging from T-ALL is that the oncogenic role of Notch1 appears to be an exaggeration of its normal functions. Notch1 is essential at multiple junctures of T cell development, including T cell specification and thymocyte proliferation at β-selection (for recent review, see [3]). By analogy, selective pressures for gains or losses of Notch activity in other cancers may also reflect some normal aspect of Notch function in the cells of origin.

3. Evidence for oncogenic Notch signaling in chronic lymphocytic leukemia and other B-cell malignancies

Early work on Notch signaling in B lineage cells focused on its antagonism of B cell development. Strong gain-of-function *NOTCH1* alleles skews the differentiation of hematopoietic progenitors towards T cell fate and away from B cell fate [4], and leads to growth arrest or apoptosis of several B cell neoplasms [5]. By contrast, notch2 is essential for murine splenic marginal zone B cell development [6], and notch1 has been implicated in B cell activation [7] and Ig secretion [8]. The possible ability of Notch to antagonize early steps in B cell development yet act positively at subsequent stages is reminiscent of the complex role of Notch in tissues such as the peripheral nervous system and the eye of *Drosophila* that arise through successive hierarchical cell fate decisions. It is thus possible that Notch may have additional uncharacterized roles in the development or function of various B cell subtypes.

Chronic lymphocytic leukemia (CLL) is an indolent but incurable neoplasm with a gene expression signature resembling that of normal memory B cells [9]. Several past reports have suggested a role for Notch signaling in CLL, but genetic evidence of Notch involvement was lacking. This changed in 2009, when focused resequencing of 43 cases of CLL by Falzetti's group identified 2 cases with *NOTCH1* exon 34 mutations leading to PEST degron deletions [10]. Subsequent study of 133 newly diagnosed CLL cases by the same group confirmed a low frequency of Notch1 PEST domain mutations (5.3%) [11]. In 2011, two unbiased whole exome studies of large European CLL cohorts also identified Notch1 gain-of-function mutations [12, 13]. In combination, these studies detected Notch1 mutations in 56 of 561 (10.0%) of newly diagnosed tumors. Notch1 mutations were associated with a worse outcome in all three of these series, and in the series from the Gaidano group [13] were associated with disease progression to large cell lymphoma and refractoriness to chemotherapy. Notch1 mutations were also frequently subclonal or undetectable at diagnosis and clonal in transformed tumors from the same patient, providing additional evidence of a link between Notch1 signaling and disease progression. Conversely, Notch1 mutations were identified in only 2 of 63 monoclonal CD5-positive B cell proliferations [14], an early stage of CLL development. Several groups have recently also reported that Notch1 mutations are enriched in CLLs associated with trisomy 12 [15–17], a common cytogenetic aberration in CLL.

Subsequently, Gascoyne's group found *NOTCH1* gain-of-function mutations in approximately 12% of mantle cell lymphoma (MCL) [18], an aggressive neoplasm usually derived from immunologically naïve mature B cells. As in CLL, most of these mutations lead to PEST degron deletions. Prior studies indicated that the *NOTCH1* locus is frequently hypomethylated in MCL cells [19], but otherwise there was little reason to suspect a role for Notch signaling in this disease. Beyond MCL, Gaidano's group also identified several diffuse large B cell lymphomas with Notch1 PEST domain mutations [13], and a recent report from Japan described a handful of diffuse large B cell lymphomas with Notch2 PEST domain mutations [20]. It is thus increasingly evident that Notch signaling has an oncogenic role in a number of B cell malignancies.

Several features of Notch1 mutations in CLL and MCL differ from those in T-ALL (Fig. 2). Virtually all of the mutations are PEST deletions, and of these roughly 90% in CLL and >50% in MCL consist of a del(CT) mutation involving codon 2514. This contrasts sharply with human and murine T-ALL, in which PEST mutations occur across a wide region (Fig. 2). Furthermore, while the NRR is the most common site of mutations in human T-ALL, and >95% of CLL and MCL have wild type NRR sequences. The Notch1 mutations in CLL and MCL thus raise several questions: 1) why are del(CT) mutations so prevalent (relative to T-ALL)?; 2) how is ICN1 generated in the absence of NRR mutations?; and 3) do Notch1 mutations reflect some normal role for Notch1 in mature human B cells?

Structure/function analyses suggest that the functional effect of the codon 2514del(CT) mutation is unlikely to differ from other PEST mutations, and indeed other Notch1 PEST mutations occur in CLL and MCL. A more likely possibility is that codon 2514 is embedded within a DNA context that is prone to spontaneous microdeletion. Of note, codon 2514 lies within the first of two 9 base pair direct tandem repeats (Fig. 2), which may make this sequence susceptible to “slippage” during DNA replication. If this is correct, codon 2514del(CT) mutations should be relatively common in all human cancers in which there is selective pressure for Notch1 gain-of-function. Consistent with this prediction, codon2514 del(CT) is the most common *NOTCH1* mutation in human T-ALL as well. The idea that DNA sequence influences the distribution of PEST mutations is also supported by a comparison of human and murine T-ALL (Fig. 2). In contrast to human T-ALL, in murine T-ALL the most common PEST mutations are generated by insertions or deletions centered on codon 2361 (codon 2372 in human *NOTCH1*), which in the mouse (but not in the human) is framed by two direct 7 base pair tandem repeats that may promote illegitimate recombination events. Del(CT) mutations also occur in murine T-ALL, but at lower frequency than in human disease, possibly because of a single base substitution in the first of the two direct repeats (Fig. 2).

How ICN1 is generated in CLL and MCL is of biologic and therapeutic importance, since the mechanism will dictate the choice of targeting strategies. CLL cells proliferate in a specialized niche found mainly in lymph nodes that is defined by the presence of “nurse cells”, which are known to express membrane-bound factors such as BAFF that stimulate CLL growth and survival; one attractive possibility is that these cells also express Notch ligands. A second possibility is that the region encoding the Notch1 NRR may be disrupted by a mutation that might be missed by whole exome sequencing, such as a deletion or rearrangement; as mentioned, this type of mutation is common in murine T-ALL and also occurs in human breast cancer (described later). Finally, genetic studies in invertebrates have shown that aberrant Notch trafficking into late endosomes leads to ligand-independent receptor activation; the contribution of this mechanism to oncogenic Notch signaling in mammalian cancer is unknown.

It will also be of interest to compare and contrast the genes and pathways that are upregulated by Notch1 in B-cell tumors and T-ALL. Major oncogenic targets of Notch1 in T-ALL include *MYC*, the PI3K/Akt pathway, and possibly the NF-kB pathway as well. Of possible relevance, EBNA2, an Epstein-Barr virus (EBV) protein that binds CSL and functionally resembles ICN, directly upregulates *MYC* in EBV-transformed B cells [21]. Expression profiling suggests that *MYC* is also a direct target of Notch1 in MCL cell lines [18], supporting the idea that Notch signaling promotes the proliferation of transformed B cells. Proliferation index predicts outcome in MCL [22], bolstering the rationale for targeting Notch1 in patients with this disease.

4. Evidence for oncogenic Notch signaling in breast carcinoma

Interest in Notch signaling in breast carcinoma is longstanding. Notch4 was originally identified as the target of a retroviral insertion in a murine mammary tumor [23], and subsequent work confirmed the oncogenic activity of ICN1 and ICN4 in murine mammary epithelium. In models using ICN1, it appears that its oncogenic effects are again mediated through the upregulation of *MYC* [24]. Other work has suggested roles for Notch in maintenance of breast cancer cells with "stem-like" properties [25, 26] and in promotion of anchorage-independent growth [27]. However, a "smoking gun" in the way of activating mutations in human breast cancer has been lacking.

This changed recently when Chinnaiyan's group identified abnormal Notch mRNAs in eight breast carcinoma cell lines and primary tumors [28]. All of these tumors were negative for expression of estrogen receptor (ER−), and 7 or 8 belonged to the so-called triple negative subgroup that is associated with a worse prognosis. The aberrant transcripts resulted from cytogenetically silent rearrangements of the *NOTCH1* (6/8) or *NOTCH2* (2/8) genes; although several of the rearrangements produce fusion genes, none appear to encode chimeric proteins. Instead, the resulting mRNAs are predicted to encode polypeptides that initiate from start codons within (Notch1) or just internal to (Notch2) the transmembrane domain, the familiar theme being the expression of Notch polypeptides lacking an NRR. *MYC* again appeared to be a downstream target of Notch in human breast cancers with Notch gene rearrangements.

Of therapeutic importance, the aberrant Notch polypeptides identified by Chinnaiyan's group will not be inhibited by antibodies directed against Notch receptor ectodomains or Notch ligands, or (in the case of the Notch2 polypeptides) even GSIs, all of which are being tested or considered as therapies in breast cancer. By contrast, agents that directly target the Notch transcription complex should be effective; such inhibitors are also in preclinical development.

5. The Flipside of the Coin: Notch as a Tumor Suppressor

Radtke and co-workers were the first to describe an increased incidence in skin cancers in conditional *notch1* knockout mice [29]. Deletion of Notch1 in the skin of one-week old mice resulted in spontaneous basal cell carcinoma (BCC)-like lesions with increased Gli2 expression in 95% of mice by 12 months of age. The incidence of papilloma formation in *notch1* null skin was also markedly increased by exposure to chemical carcinogens plus phorbol ester, with a small proportion progressing to either BCC or squamous cell carcinoma (SCC). Proweller et al. next noted that expression of a specific inhibitor of the Notch transcription complex, dominant negative MAML1 (DN-MAML), in murine skin led to the development of cutaneous SCC but not BCC [30]. Differences in tumor phenotypes observed in these two models may have been related to the degree of Notch loss-of-function, as multiple Notch receptors are expressed in keratinocytes and DN-MAML is a pan-Notch inhibitor that produced a much more severe preneoplastic phenotype consisting of cutaneous hyperplasia and alopecia. Subsequent work showed that human keratinocytes expressing oncogenic RAS and deficient in Notch signaling form SCC in immunocompromised mice [31], setting the stage for the genomic discoveries described below.

A number of tumor suppressive mechanisms have been proposed for Notch in the skin. Dotto's group identified several Notch targets that may mediate pro-differentiation and anti-growth effects, such as the cell cycle regulator p21 [32] and the transcription factor Irf6 [33]. Crosstalk between Notch and other pathways linked to skin carcinogenesis, including Ras, NF-kB, Wnt, and Hedgehog, have also been described in various contexts, each of which could contribute to a cell autonomous tumor suppressive activity. By contrast, Kopan and

co-workers have postulated that epidermal barrier defects caused by Notch loss-of-function produce a chronic cutaneous inflammatory state that promotes cancer development through factors released from dermal stromal and inflammatory cells [34]. Thus, it is possible loss of Notch function contributes to skin carcinogenesis through both cell autonomous and non-cell autonomous effects.

While the mechanism(s) remain to be defined, abundant genetic evidence emerging in the past year is consistent with Notch having a tumor suppressive role in multiple types of human SCC (Table 2). Whole exome deep sequencing by two groups independently identified likely loss-of-function mutations in a least one Notch signaling component in roughly 15% to 20% of head and neck SCC (Fig. 3) [35, 36]. In parallel, in studies also using whole exome deep sequencing, we noted the presence of at least one putative loss-of-function mutation involving either *NOTCH1* or *NOTCH2* in 19 of 26 primary cutaneous SCC or derived cell lines [37]. Most of these mutations were truncating or likely to be structurally disruptive, and one mutation predicted to be "benign" based on polyphen-2 analysis, a R1594Q substitution in the Notch1 NRR, interfered with ligand-mediated receptor activation in cell-based assays [37]. Several other mutations identified in our series were also confirmed to produce loss-of-function, including a point substitution in intracellular Notch1 that abrogates binding of ICN1 to RBPJ, and a point substitution in EGF repeat 12 that disrupts ligand-binding [37, 38]. Despite the preceding murine data, no *NOTCH1* or *NOTCH2* mutations were seen in 8 BCC [37]; sequencing of additional tumors will be needed to see if this difference is real or merely a product of small sample size.

These results raise the question of whether Notch signaling is disrupted in all SCCs in one way or another. Of note in this regard, MAML1 was originally identified as a human papilloma virus 16 (HPV16) E6-binding protein. Targeting of the Notch signaling pathway by high-risk human papilloma viruses such as HPV16 (a possibility yet to be thoroughly explored) would further solidify the fundamental role of the Notch signaling pathway as a tumor suppressor in human SCC. However, Agrawal et al. [36] noted several HPV-positive tumors in their series of head and neck cancers that nevertheless exhibit Notch gene mutations, and the contribution of HPV interference with Notch to squamous cell carcinogenesis requires further investigation.

A smaller fraction of lung SCC have acquired Notch receptor mutations [37], and the importance of Notch as a tumor suppressor in this other type of SCC is unsettled at this time. Conversely, a paper relying on focused Sanger resequencing reported that gain-of-function Notch1 PEST domain mutations were found in roughly 10% of human lung carcinomas [39], but deep sequencing has yet to confirm this association.

6. Other Possible Tumor Suppressive Roles for Notch

Aifantis' group reported recently that knockout of nicastrin, a component of g-secretase, produces a myeloproliferative disorder in mice [40]. This same report identified several mutations involving Notch pathway components in human chronic myelomonocytic leukemia, including a truncation mutation in MAML1 predicted to yield a dominant negative polypeptide. Loss-of-function mutations in Notch receptors were not detected, however, and since γ-secretase cleaves many substrates besides Notch and MAMLs interact with multiple signaling pathways, these phenotype could stem from "off-Notch" effects. Sequencing of the genomes of human myeloproliferative disorders will soon reveal the extent of Notch's involvement as a tumor suppressor in this type of human malignancy.

Other work has raised the specter of a tumor suppressive role for Notch signaling in vascular tumors. Notch and VEGF co-regulate many facets of vasculogenesis, angiogenesis, and endothelial cell homeostasis. Rats treated chronically with DLL4 blocking antibody develop

subcutaneous vascular tumors and hepatic vascular abnormalities associated with endothelial cell activation [41]. Furthermore, conditional *notch1* deficiency in mice is also associated with vascular proliferations, most commonly in the liver [42]. It will thus be of interest to determine if deep sequencing of human angiosarcomas or other human vascular neoplasms reveals Notch loss-of-function mutations.

7. Concluding Remarks

Recent discoveries emphasize the multifaceted role of Notch signaling in cancer. Notch is an increasingly attractive therapeutic target in multiple human malignancies, including some not discussed here, such as melanoma and ovarian cancer. Notch signaling also has roles in the immune system that suggests opportunities for therapeutic intervention in other cancer-relevant conditions, such as graft versus host disease. Conversely, given the frequency of SCC in light-skinned sun-exposed populations, it is likely that the tumor suppressive role of Notch signaling supersedes its oncogenic role, a relationship that will further complicate attempts to treat patients with drugs or antibodies that chronically inactivate Notch. This includes the use of GSIs in patients with Alzheimer disease, which has been attributed to pathogenic peptides generated by cleavage of amyloid precursor protein by γ-secretase. Notably, a phase III trial of the GSI semagacestat was stopped in 2010, mainly due to worsening of cognitive function, but also because the treatment group had an increased incidence of skin cancers. Going forward, this risk will need to be calculated into any attempt to target oncogenic Notch signaling with broad-spectrum inhibitors such as GSIs.

On a basic level, the divergent context-dependent effects of Notch signaling in cancer cells speaks to a fundamental question: why are certain genes commonly mutated in some cancers but not others? Notch1 takes this question to a new level by being the most frequently mutated oncoprotein in one human cancer (T-ALL) and one of the most frequently mutated tumor suppressors in a second (SCC). Recently developed whole genomic approaches for understanding how factors regulate transcriptomes globally, such as ChIP-Seq, now provide a means for elucidating the basis for the double-edged effect of Notch signaling in various cancers genome-wide. Together with detailed study of Notch/genome interactions during normal development, these studies should lead to a fuller understanding of Notch's cell autonomous roles in cancer.

Highlights

- We present new findings linking activating mutations in Notch1 to B cell neoplasms
- We discuss the discovery of Notch gene rearrangements in breast cancer
- We review the discovery of loss-of-function mutations in Notch receptors in squamous cell carcinoma

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Abbreviations

GSI	γ-secretase inhibitor

NRR	negative regulatory region
EGF	epidermal growth factor
LNR	Lin12/Notch repeat
ADAM	a disintegrin and metalloprotease
DSL	Delta-Serrate-Lag2
ICN	intracellular domain of Notch
MAML	Mastermind-like
RBPJ	recombining signal binding protein for immunoglobulin kappa J region
CLL	chronic lymphocytic leukemia
T-ALL	T-cell acute lymphoblastic leukemia/lymphoma
MCL	mantle cell lymphoma
SCC	squamous cell carcinoma

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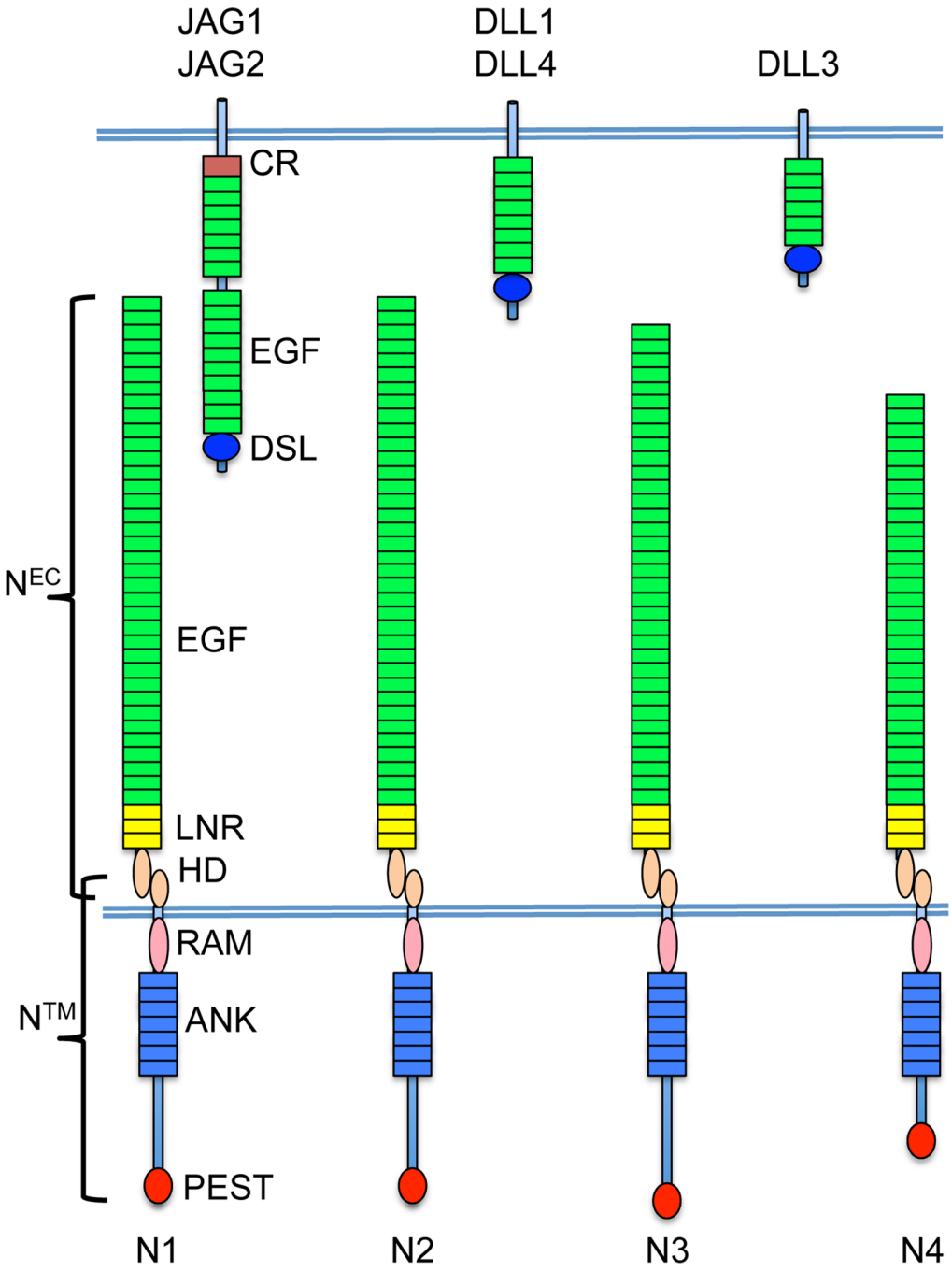

Figure 1.

Structure of mammalian Notch receptors and ligands. Mammals express 5 Notch ligands, four of which activate Notch receptors (JAG1, JAG2, DLL1, and DLL4), and one of which (DLL3) may function as a decoy. Ligands have an N-terminal DSL domain, variable numbers of EGF repeats, and in the case of Serrate-like ligands (JAG1 and JAG2) a juxtamembrane cysteine-rich domain (CR). Mammals have four Notch receptors, Notch1–4, comprised of non-covalently associated extracellular (N^{EC}) and transmembrane (N^{TM}) subunits. N^{EC} is comprised of 29–34 EGF repeats, 3 Lin12/Notch repeats (LNRs), and the N-terminal portion of the juxtamembrane heterodimerization domain (HD), while N^{TM} is comprised of a RAM domain, 7 iterated ankyrin repeats (ANK) repeats, an structurally

divergent unfolded region with variable transcriptional activation domain function (greatest in Notch1, least in Notch4), and a C-terminal PEST degron domain.

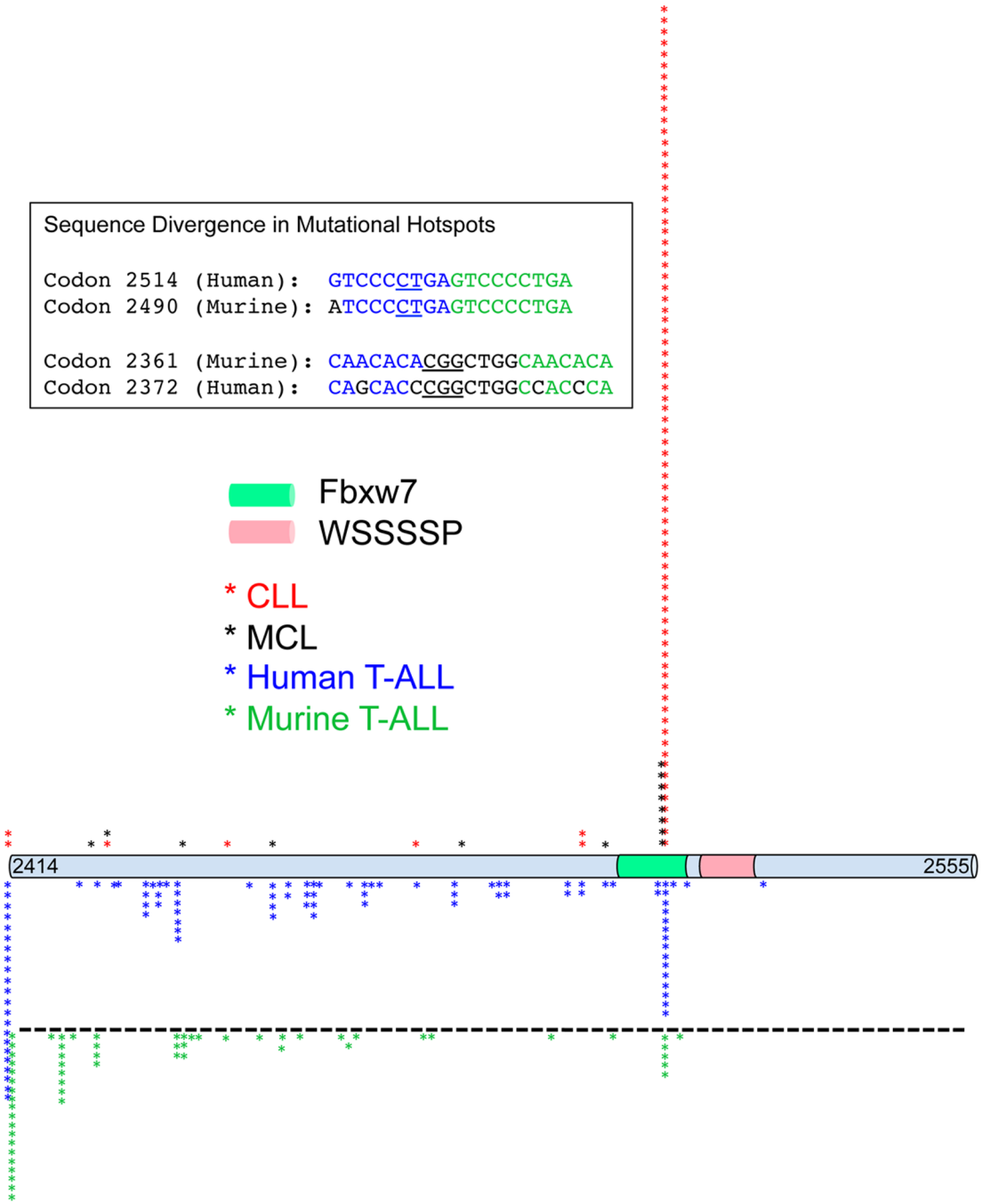

Figure 2.

Distribution of Notch1 PEST domain mutations in lymphoid cancers. Positions of nonsense and frameshift mutations in human chronic lymphocytic leukemia (CLL) [11–13], human mantle cell lymphoma (MCL)[18], and human [43–46] and murine T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) [47, 48] are superimposed on the human C-terminal Notch1 amino acids 2414 and 2555, which includes a Fbxw7 E3-ligase target sequence and the sequence WSSSSP, both implicated in degradation of activated Notch1. Murine Notch1 mutations are aligned according to the homologous amino acid sequences of human Notch1. Disruptive mutations N-terminal of amino acid 2414 of human Notch1 are collected together and thus appear as a single peak. The inset box shows the murine and

human genomic sequences around mutational hotspots in human lymphoid tumors (codon 2514) and in murine T-ALL (codon 2361).

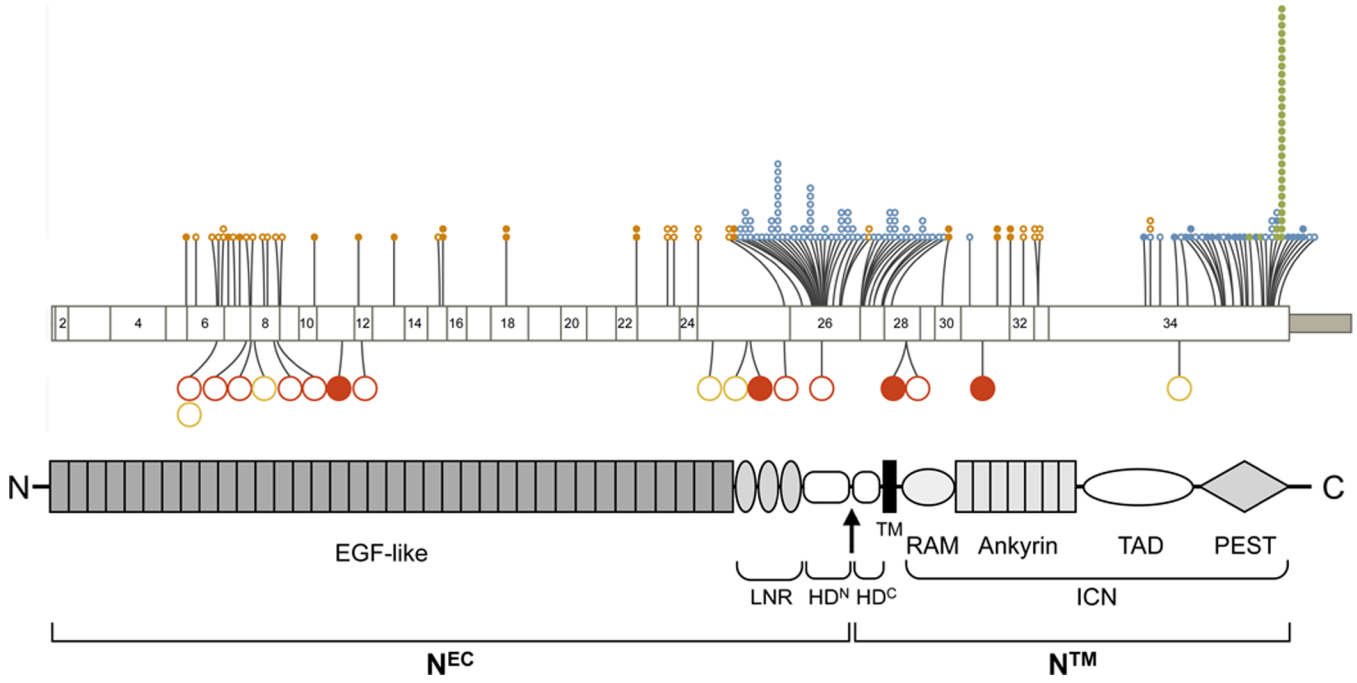

Figure 3.

Distribution of cancer-associated missense (open circle) and nonsense (closed circle) mutations in *NOTCH1*, organized by exon and protein domain. Selected activating mutations in T-ALL (blue) and CLL (green) are compared with putative loss-of-function mutations for head and neck SCC (orange), cutaneous SCC (red) and lung SCC (yellow).

Table 1

Activating Notch Mutations in Human Cancers

	NOTCH1	**Other Notch genes**	**Somatic aberration**	**Comments**
T-ALL	~60% in human T-ALL	?NOTCH3 (rare)	In-frame NRR mutations and C-terminal PEST degron deletions	Mutations cause ligand-independent activation (NRR) or enhance protein half-life (PEST)
CLL	5–12%	unknown	PEST degron deletions (>90% codon2514(del(CT))	Associated with transformed and refractory CLL, absence of somatic hypermutation, trisomy 12
MCL	~10%	Confined to *NOTCH1*	PEST degron deletions (>50% codon2514(del(CT))	*NOTCH1* locus also hypomethylated in MCL
Breast adenocarcinoma	<5%	*NOTCH2* fusion genes also detected	Activating gene fusions	All rearrangements in ER- cancers, functionally validated

Table 2

Loss-of-function Notch mutations in human cancer.

	NOTCH1	**Other Notch genes**	**Somatic aberration**	**Comments**
SCC (skin)	60–70%	*NOTCH2* > 25%, isolated *NOTCH3* and *NOTCH4* truncations	Frequent 5' nonsense mutations, disruptive in-frame sequence variants	Functionally validated missense mutations, biallelic and heterozygous mutations identified, some predicted to have dominant negative activity
SCC (head and neck)	15–20%	Confined to *NOTCH1*		*IRF6* and *TP63* also recurrently mutated
SCC (lung)	5–10%	undefined		TCGA sequencing ongoing