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NEWS AND COMMENTARIES

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Activated Notch takes center stage in T-cell leukemogenesis

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A convincing new study published in *Science* has upgraded the status of *NOTCH1* from bit-part player in acute T-cell lymphoblastic leukemia (T-ALL) to that of a central participant in the development of this aggressive cancer.[1]

Previously, only T-ALL tumors carrying the rare t(7;9) translocation were thought to result from the activation of *NOTCH1*.[2,3] However, Weng *et al* have now found activating *NOTCH1* gene mutations in the majority of samples from patients with acute T-cell lymphoblastic leukemia (T-ALL).

Since the initial discovery of mammalian NOTCH1 as the target of the t(7;9) translocation, the normal biology of the four mammalian Notch receptors (NOTCH1–4) has been extensively characterized.[4] During transit to the cell surface, mammalian NOTCH1 is cleaved by a furin-like protease to form heterodimeric receptors consisting of noncovalently associated extracellular and transmembrane subunits. The extracellular subunit consists of EGF-like repeats that bind DSL (Delta-Serrate-Lag2) ligands; three iterated Notch/Lin-12 repeats (LNR); and a conserved ~100-amino-acid region that interfaces with the extracellular portion of the transmembrane subunit (Figure 1). These last two regions comprise the heterodimerization (HD) domain, which is sufficient to maintain a stable association between the extracellular and intracellular subunits.[5] The intracellular portion of the transmembrane subunit mediates Notch signaling and contains a RAM domain, seven iterated ankyrin repeats, and a C-terminal PEST sequence thought to be important for protein stability.

Ligand binding initiates a series of enzymatic cleavages that activate Notch signaling. First, a metalloprotease cleaves the extracellular subunit, leaving a short-lived membrane-bound form of the transmembrane subunit. Second, a multiprotein complex with γ-secretase activity recognizes a cleavage site within the transmembrane domain, thereby releasing the intracellular domain of Notch (ICN), which translocates to the nucleus to activate transcription.

Ligand-independent activation of Notch (eg ectopic expression of activated forms of Notch) leads to gain of function phenotypes in organisms ranging from *Caenorhabditis elegans* to humans. Moreover, when expressed in hematopoietic progenitors, activated Notch isoforms drive thymic-independent T-cell development in the bone marrow and induce T-ALL.[6–8] These tumors resemble the human T-ALLs associated with the t(7;9) translocation. In addition, previous studies from the Aster group showed that cell lines derived from the Notch-induced murine T-ALLs and human t(7;9) T-ALL remain dependent on persistent Notch signaling for growth and survival.[9]

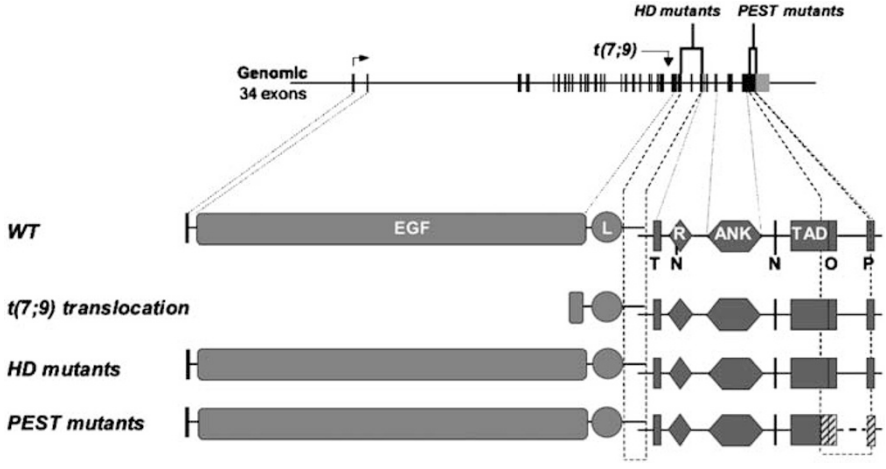

Figure 1 Activating *NOTCH1* mutants in the HD (heterodimerization) and PEST (proline, glutamic acid, serine and threonine-rich) regions. The genomic organization of the human/mouse NOTCH1 gene spanning ~50 kb is shown. The hatched area represents variability in the sites of PEST region. EGF = epidermal growth factor-like repeats, L = lin12-like repeats, T = transmembrane domain, R = RAM = RBP-Jk/CSL associating motif, N = putative nuclear localization domain, ANK = ankyrin-like repeats, TAD = transactivating domain, O = OPA = glutamine-rich region, P = PEST region. Although the t(7;9) looks similar to the HD mutations, it lacks the N-terminal signal peptide, causing the vast majority of this protein to remain intranuclear with the result that γ-secretase inhibitiors do not affect this protein. Adapted with permission from Zweidler-McKay and Pear.[14]

In this new study, Aster and colleagues used a γ-secretase inhibitor to screen 30 human T-ALL cell lines for dependency on Notch signaling. Five lines underwent cell cycle arrest in the presence of γ-secretase inhibitors. Sequencing of *NOTCH1* identified two sets of mutations in cis: one set within the HD domain and another that causes partial or complete deletions of the PEST sequence. Approximately 55% of the 96 primary pediatric human T-ALLs had at least one mutation in either the HD domain or the PEST sequence and 20% had mutations in both sites. Independently, the two sets of *NOTCH1* mutations exhibited weak to modest activation of transcriptional reporters, but in combination, they strongly activated these reporters. Intriguingly, *NOTCH1* mutations were found in association with oncogenes of all the major molecular T-ALL subtypes including tumors associated with expression of *TAL-1*, *HOX11*, and *LYL1* as well as the minor subtypes such as *HOX11L2*, *MLL-ENL* and *AF10-CALM*. Further work has shown a similar incidence of *NOTCH1* mutations in adult T-ALL (JC Aster, personal communication).

So how do HD and PEST mutations activate NOTCH1? Recent studies from the Blacklow group found that the HD domain was conserved and required to stabilize the association of the extracellular and intracellular subunits, which prevents ligand-independent cleavage.[5] Moreover, in worms, mutations in the presumed HD domain of the Notch homologs glp-1 and lin-12 lead to gain-of-function Notch phenotypes that specify cell fate or proliferation.[10,11] These data have prompted the Aster group to suggest that HD domain mutations destabilize the association of the extracellular and transmembrane subunits, leading to ligand-independent Notch proteolysis and release of ICN. The authors also predict that the loss of PEST sequences would reduce the rate of NOTCH1 turnover and thereby elevate NOTCH1 activity. Certainly in mice targeting of the *NOTCH* C-terminus seems to shorten T-ALL latency.[12,13] However, these predictions await formal proof and ligand-independent activation through PEST mutations has not been ruled out.

Clearly, these new findings place NOTCH1 signaling at the center of T-ALL pathogenesis and therapy. However, whether the main effects of NOTCH1 are to promote/maintain a T-cell program or to affect proliferation and/or survival remains to be determined. Similarly, the precise signaling pathways that NOTCH1 influences in T-cell leukemia are unknown: the consistent involvement of NOTCH1 in many subtypes of T-ALL suggests that it has the potential to influence multiple signaling pathways. As NOTCH1 has not been yet been demonstrated to be important in myeloid or B-cell malignancies, the transforming pathways are likely to be specific to developing T cells within hematolymphoid cells. It will be important to delineate how *NOTCH1* cooperates promiscuously with many other genes to drive T-ALL development. It is also surprising that only NOTCH1 has been associated with the activating mutations in human T-ALL, as constitutive expression of the intracellular domains of Notch2 and Notch3 cause T-ALL in mice and cats.[14] The basis for the NOTCH1 selectivity among the four mammalian Notch receptors is unknown.

Given the high prevalence of activating *NOTCH1* mutations, NOTCH1 should be an attractive pharmacological target for the treatment of T-ALL. γ-Secretase inhibitors initially developed for possible use in Alzheimer's patients offer a rational, molecularly targeted therapy. However, Weng *et al*'s data must be interpreted cautiously. Rather than killing tumor cells, γ-secretase inhibitors primarily suppressed growth of the T-ALL cell lines (ie was cytostatic). Therefore, in clinical practice, γ-secretase inhibitors might need to be combined with conventional chemotherapy to effectively destroy tumor cells. Since cytostatic effects might make tumors less responsive to treatments that target proliferating cells, developing effective drug combinations might not be straightforward. Furthermore, a number of the T-ALL cell lines in the authors' panel harbored activating *NOTCH1* mutations, but were insensitive to γ-secretase inhibition *in vitro*. The mechanism and prevalence *in vivo* of γ-secretase resistance is unclear and raise a warning flag that *NOTCH1* mutations might not always be required for maintenance of established tumors. Thus, it will be important to ascertain the response of primary T-ALL cells to Notch withdrawal *in vivo* and in murine models.

Where does this work take us? The authors propose that dysregulated Notch signaling may be more prevalent than we realize, as some of their γ-secretase-dependent cell lines do not bear *NOTCH1* mutations. They speculate that other components of the Notch signaling pathway might be activated in these cells. More broadly, Weng *et al*'s success in screening for activating *NOTCH* mutations through γ-secretase sensitivity is an invitation to other researchers to perform similar analyses in other tumor types. Recent data that suggest roles for Notch in diverse human cancers, including carcinomas of the breast, pancreas, and prostate, and central nervous system neoplasms, provide a compelling rationale for functional screening, which could greatly expand the potential therapeutic use of Notch inhibitors■

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Research Network

EUROGLYCANET: a European network focused on congenital disorders of glycosylation

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The congenital disorders of glycosylation (CDG) are an emerging group of inborn errors of metabolism. Most of these genetic defects result in severe disease, mental retardation and physical handicap. EUROGLYCANET, a European network that focuses on the advancement of research, diagnosis and treatment of CDG, was created in 1999 and has been supported by the European Commission since 2000 (see Figure 1).

In this network, clinical and basic scientists collaborate in order to identify novel types of the disease and try to promote early diagnosis. These research activities are grafted onto a central database and patient sample repository. The samples circulate among the different expert laboratories – a process for which the term 'carousel testing' was coined. The network also aims to provide the basic diagnostic tools to physicians all over Europe by establishing referral laboratories in national centres. The ultimate goal of the project is to be able to precisely diagnose all cases of CDG, to get a complete inventory of the enzymatic defects that cause protein glycosylation defects and to extend the therapeutic tools available to treat CDG.

Glycosylation is the most complex type of biomolecule modification that occurs in living organisms.[1] The glycosylation pathway starts as a co-translational process in the *endoplasmic reticulum* (ER). A 'standard' oligosaccharide or glycan, containing 14 sugar residues, is assembled in the ER and transferred onto the nascent protein, after which the glycoprotein is transported to the Golgi apparatus. During its transit through the Golgi, the glycan structures are gradually modified into more complex, sometimes much specialised structures. This modification process is organised like in an 'assembly line', whereby the different glycosyltransferases act in a very strict order, partly as a result of their compartmental localisation in the Golgi. This process leads to the production of thousands of different glycoproteins with a myriad of different glycan chains.

So, inborn errors of glycosylation or CDG are typically multisystem diseases, with a broad spectrum of symptoms that include mental retardation and severe developmental delay, structural abnormalities of the central nervous system, cardiac defects, malformations, hormonal dysregulation, coagulation problems and peripheral neuropathies.[2] CDG causes a high morbidity and a significant mortality.

Relatively simple laboratory tests that detect abnormal glycosylation in serum proteins can be used to diagnose most patients with CDG. However, expert enzymatic, biochemical or molecular investigations are required to identify the underlying glycosylation defect. So the expert diagnostic services of the laboratories that are members of the network have