Comment on Sulis et al, page 733

Fifty ways to Notch T-ALL

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The fireworks from the spectacular emergence of Notch as the protagonist in the etiology of T-ALL are not likely to end any time soon. Indeed, in this issue of Blood, Sulis and colleagues describe yet another sophisticated mechanism by which ligand-independent activation of NOTCH1 is achieved in T-ALL. NOTCH1-activating mutations mark more than half of all T-ALL cases, underscoring the fundamental role of aberrant NOTCH1 signaling in this disease.

Activation of NOTCH signaling by Delta-like ligands is required for commitment of early thymic progenitors to the T-cell lineage as well as for the double-negative 3a (DN3a) to DN3b thymocyte transition, and entry to β-selection. It is therefore not surprising that the aberrant, ligand-independent activation of Notch is a hallmark of human T-cell lymphoblastic leukemia (T-ALL).

NOTCH receptors are type-I transmembrane glycoproteins that regulate cell growth, differentiation, and tissue homeostasis in multiple systems. NOTCH signaling is tightly regulated by the members of the Delta, LAG-2 Serrate family of NOTCH ligands binding to the extracellular domain of the mature receptor. Initial proteolytic cleavage of the NOTCH precursor protein within the extracellular domain yields an extracellular and a transmembrane subunit that are held together by a heterodimerization (HD) domain (see figure). NOTCH receptor heterodimers further stabilize themselves in a resting state through 3 LIN-12/NOTCH repeats (LNRs), which seem to engulf the HD domain and shield the S2 site from proteolytic cleavage by ADAM-type metalloproteases. S2 cleavage is triggered when the receptor binds to its ligand, and is a prerequisite for subsequent cleavage by γ-secretase at the S3 site. This final step releases intracellular Notch (ICN) from the membrane and allows it to translocate to the nucleus, where it converts CSL repressor into activator complexes by displacing corepressors and attracting coactivators.

In human T-ALL, activating NOTCH1 mutations are concentrated in exons 26 and 27, encoding the HD domain, and in exon 34, encoding the PEST domain, which is important for proteasomal degradation of ICN1. Thus far, the described mutations can be grouped into 3 categories. HD mutations comprise single amino acid substitutions and small in-frame deletions and insertions that allow for ligand-independent deletions and insertions that allow for ligand-independent proteolytic cleavage of S2 and γ-secretase–dependent S3 cleavage, ultimately releasing ICN1. This can be achieved either by destabilizing the HD-LNR interaction, resulting in increased dissociation of the heterodimer (class 1 mutants), or by displacing the S2 site out of the protective reach of the HD-LNR complex and exposing it to proteolytic cleavage (class 2 mutants). PEST mutations, in contrast, encode premature stop codons. Loss of the PEST domain results in increased ICN1 levels due to reduced proteasomal degradation.

Sulis and colleagues have now identified a fourth family of NOTCH1-activating mutations in T-ALL accounting for a subset of leukemias that show elevated ICN1 but none of the previously characterized mutations in exons 26, 27, or 34. In an elegant biochemical approach, the authors characterize a novel group of mutations in the extracellular juxtamembrane domain of NOTCH1. These juxtamembrane extension mutants (JEMs) distance the entire HD-LNR complex from the membrane (see figure), and presumably allow ligand-independent proteolytic processing of S2. They are generated by internal tandem duplications in the 3’ end of intron 27 and/or the proximal region of exon 28. Thus, they do not alter the spacing within the HD-LNR complex, but rather shift the entire complex away from the membrane. In fact, the authors show that JEMs do not alter HD-LNR complex stability. Moreover, the activity of JEMs is critically dependent on the length of the inserted sequence, that is, the increased distance from the membrane rather than the sequence of the insertion per se.

The authors offer 2 possibilities to explain why the maintenance of the HD-LNR complex in close proximity to the membrane is strictly required for avoiding spontaneous metalloprotease cleavage of NOTCH1. They hypothesize that “the HD-LNR complex interacts with membrane proteins that contribute to hold this structure together and that this interaction is disrupted upon displacement of the HD-LNR complex away from the membrane” (page 739; see figure panel B). They also suggest the alternative possibility that “the proximity to the membrane restrains the ability of proteases to access the S2 cleavage...
site located within the HD-LNR complex, and that displacement from the cell surface makes this sequence more accessible for pro tease cleavage” (page 739; see figure panel A). In conclusion, the authors have identified a novel family of NOTCH1-activating mutations that result in aberrant levels of ICN1, further expanding our understanding of the important molecular parameters that control Notch activity.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Comment on Ellis et al, page 741

Taking a SNPshot of t-AML

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In this issue of Blood, Ellis and colleagues report on the interaction of SNPs in the p53 tumor suppressor pathway and in the MDM2 309 locus in susceptibility to therapy-related AML.

Genetic variants are associated with disease susceptibility.1,2 Of the genetic variants, single-nucleotide polymorphisms (SNPs) lend themselves to interrogation in population-based studies. As reported in this issue of Blood, Ellis and colleagues studied 2 separate, large cohorts of therapy-related acute myeloid leukemia (t-AML) patients. Because only a subset of all patients treated with cytotoxic chemotherapy or radiation develop t-AML, it was hypothesized that these individuals may be predisposed due to constitutional genetic variations in DNA damage-response pathways. Although several candidate genes have been previously implicated, Ellis et al focused on the p53 tumor suppressor pathway, as this transcription factor mediates cell-cycle arrest, cell senescence, and apoptosis, and is often lost or mutated in t-AML. They also examined a common SNP (SNP309) of MDM2, a ubiquitin E3 ligase, which negatively affects the stability of p53 and has been examined previously in other series of leukemia patients.3 As reported in their article, an arginine (Arg) at TP53 codon 72 predisposes cells to apoptosis, whereas a proline (Pro) mediates cell-cycle arrest. At the MDM2 SNP309, a G allele indicates high binding ability of SP1 transcription factor, which increases levels of MDM2, thereby decreasing p53 expression. In contrast, a T allele allows increased p53 function.

The cohort of t-AML patients from the University of Chicago was selected because of availability of Epstein Barr virus–transformed lymphoid lines from which DNA could be extracted, whereas peripheral blood DNA was available from the cohort of patients studied from the United Kingdom. A total of 171 cases were studied. It was found that neither p53 nor MDM2 variants by themselves were associated with t-AML risk, but there was an interaction that influenced susceptibility. The figure illustrates the models proposed for these interactive influences. Control cohorts were used to determine that there was not a bias in baseline frequencies of the SNPs, and this SNP interaction was not observed in de novo AML cases. The same interactive influence was noted in those treated with chemotherapy and those who acquired abnormalities of chromosomes 5 or 7. Only TP53 Pro/Pro was associated with increased risk of t-AML in those who received chemotherapy alone. No significant effects on disease latency were noted. The MDM2 TT genotype appeared to offer a protective effect in younger women.

Although this study used 2 relatively large cohorts of t-AML patients, it would have benefitted from the melding of differing methodologies and DNA sources (although these were well-controlled for to reduce bias, and genotype distributions appeared comparable between University of Chicago and United Kingdom control subjects). The 2 series used different means of case selection/identification and different treatment regimens. Also, given that multiple therapeutic regimens were utilized, the effect of SNP interaction as related to exposure to a single agent or combination regimen on development of t-AML could not be determined. Nonetheless, this study demonstrates that interrogating biologically rational interactions between SNPs may be important in determining the risk of susceptibility to disease. Such interactions might also influence the clinical course of disease or define genetic variations that predict different toxicities and efficacies of available treatments.

The 2 SNPs examined in this work are no doubt only a snapshot of the total picture of susceptibility to therapy-related AML, but studies such as this are a beginning to improve our understanding of genetic susceptibilities. If confirmed in prospectively analyzed cohorts or other large retrospective cohorts of t-AML, these markers of therapy-related AML susceptibility might

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<th>Model 1</th>
<th>MDM2 309 TT and TP53 72 Arg/Arg</th>
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<tr>
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<td>MDM2 309 GT or GG and TP53 72 Pro/Arg or Pro/Pro</td>
<td>Decreased t-AML risk</td>
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MDM2 and TP53 interaction in t-AML. Data regarding interactions between MDM2 309 and TP53 72 alleles were consistent with double-homozygous state TT and Arg/Arg, or any genotype with at least one MDM2 SNP 309G and one TP53 codon 72 Pro resulting in increased risk of t-AML. Any TP53 Pro-containing genotype with MDM2 TT was protective.
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