Notch, a T-ALL order

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In this issue of Blood, Demarest et al demonstrate that maintenance of T-cell acute lymphoblastic leukemia (T-ALL) tumors requires continued expression of activated Notch1 while c-Myc expression is expendable for tumor maintenance.1

The data reported in this paper use a mouse model system where either the activated form of Notch1 (Notch1IC) or c-Myc is under the control of the Tet operator and inducible by removal of doxycycline from the drinking water. Induction of either Notch1IC or c-Myc is sufficient to drive the formation of T-cell leukemias that resemble human T-ALL. The authors have also used retroviral expression vectors that express either Notch1IC or c-Myc to transfer into lethally irradiated syngenic mice. These data allow the investigators to question how activation of Notch1 or c-Myc contributes to tumor development.

Several proto-oncogenes, including LMO1/2, TAL1/2, HOX11, LYL1, Notch1, and c-Myc, have been implicated in the development of T-ALL (reviewed in Teitell and Pandolfi). Particularly relevant to the data reported by Demarest et al, activating mutations in Notch1 are found in >60% of human T-ALL and introduction of these mutations in murine hematopoietic stem cells by retroviral infection leads to T-ALL in 100% of animals transplanted with these cells.3,4 These data suggest that activating mutations in Notch1 are a dominant event in the development of T-ALL in humans and mice and have led investigators to question how activation of Notch1 contributes to tumor development. An emerging consensus is that one major function of Notch is the regulation of cell growth. Suppression of Notch, ensuing growth arrest could be rescued by introduction of a gene encoding one of these downstream targets. The observation that inhibition of γ-secretase and blockade of Notch activation leads to growth arrest and the well-known link between c-Myc and cell-cycle progression, coupled with data demonstrating overlapping gene expression profiles of Notch or c-Myc in leukemia cells, suggest that Notch and c-Myc might compensate for each other in maintaining tumors. To test this hypothesis, the authors of this study used the inducible mouse models described above.

In addition to their main finding that Notch1 (but not c-Myc) is required for tumor maintenance, Demarest and colleagues show that expression of c-Myc has no effect on tumor latency in Top-Notch mice; however, expression of Notch1IC greatly enhances time to tumor formation in Tet-o-Myc mice (110 days for Tet-o-Myc vs. 42.62 days for Tet-o-Myc + Notch1IC). Earlier data from this group provided compelling evidence that Notch suppresses the p53 pathway.11 Others have demonstrated that c-Myc–induced tumorigenesis requires inactivation of p53, presumably to inhibit p53-dependent apoptosis. These observations led the authors to postulate that Notch1 drives T-ALL development and tumor maintenance by sustaining cell-cycle progression while inhibiting p53–mediated apoptosis. In support of this model, in Top-Notch/c-Myc tumors, p53 expression is extinguished; however, when Notch1IC is turned off by doxycycline, p53 protein levels are up-regulated suggesting tumor regression is mediated by p53–dependent apoptosis. The authors use this evidence to propose that the dominance of Notch1IC over c-Myc can be explained, at least in part, by the ability of Notch1IC to suppress p53–mediated apoptosis. While the data support this hypothesis, it would be useful to directly test it by precisely modulating p53 levels in Top-Notch/c-Myc tumors.

The results presented by Demarest et al advance our understanding of the role played by Notch1IC in the development of T-ALL. In particular, the data indicate that inhibition of γ-secretase, which blocks Notch activation, is likely to block cell proliferation and p53 driven apoptosis. Recent data from the Ferrando laboratory suggest that many instances where
T-ALL cells escape growth arrest are accompanied by mutational inactivation of the tumor suppressor PTEN. Inactivation of PTEN directly results in activation of the PI3K-Akt-mTOR signaling pathway, thus bypassing Notch-induced activation of this important cell growth pathway. Therapies that combine γ-secretase inhibitors with inhibitors of p38-mediated apoptosis as well as the PI3K-Akt-mTOR pathway are likely candidates to treat refractory T-ALL. Future research is likely to explore the relevance of the insights gained from the mouse model presented in this report to human T-ALL. In addition, an exploration of the ability to transplant tumors from animals where either Notch or c-Myc expression is extinguished may provide insights concerning the role of these genes in the maintenance of tumor initiating cells.

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Comment on Zhong et al, page 2924

A new face of BCL-2 inhibition in CLL

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In this issue of Blood, Zhong and colleagues report on a peptide that is selectively toxic to chronic lymphocytic leukemia (CLL) cells through perturbation of cytoplasmic calcium levels via disruption of the BCL-2:IP3R interaction.1

Cytoplasmic calcium levels are largely controlled by uptake and release from the endoplasmic reticulum (ER). Calcium levels can fluctuate dramatically over time in the form of discrete oscillations of cytoplasmic Ca2+ concentrations. These oscillations govern many important cellular processes, including proliferation and programmed cell death. An important protein controlling the pulsatile release of calcium is the inositol 1,4,5-triphosphate receptor (IP3R).2 It has been shown that the BCL-2 family of proteins, best known for controlling mitochondrial permeabilization during apoptosis, also controls cytoplasmic calcium levels.3 BCL-2 itself is present at the ER, which usually exists in very close conjunction with the mitochondria. BCL-2 can act to dampen high amplitude Ca2+ oscillations that can induce apoptosis. The observation of a complex between IP3R and BCL-2 affords the critical link between BCL-2 and physiologic control of calcium levels.4-5

In no malignancy is BCL-2 more consistently expressed at high levels than in CLL. In fact, CLL has been shown to be a consistently BCL-2-dependent disease.6 Most of the study of BCL-2 function in CLL has focused on the classic BCL-2 function of binding proapoptotic BCL-2 family members by their BH3 domain, sequestering them, and preventing execution of their pro-death function. In these circumstances, where “primed” BCL-2 is antagonized by a BH3 mimetic small molecule, such as ABT-737 (or ABT-263, the clinical derivative), the proapoptotic molecules are released to commit the CLL cell to death. This type of death relies on permeabilization of the mitochondrial outer membrane by BAX or BAK, pro-death BCL-2 family proteins. CLL remains an active area of clinical investigation of BH3 mimetic small molecules.

Zhong et al focus on a different facet of BCL-2, the BH4 domain that is involved in the interaction with IP3R. Using an oligopeptide derived from a site on IP3R found to be involved in binding BCL-2, the authors had previously demonstrated the ability to disrupt the BCL-2:IP3R complex and alter calcium signaling.7 This current report is noteworthy in two ways: first, it reports a modification of the peptide that increased cytoplasmic calcium concentrations; and second, it finds that CLL cells are selectively susceptible to death induced by the calcium signaling induced by the peptide. The original peptide was linked to the HIV TAT peptide to foster intracellular penetration via pinocytosis. However, after cell entry, the peptide was subject to proteolytic degradation. Changing 2 aspartic acid residues to alanines removed key protease sites, allowing for greater intracellular accumulation. This is reflected in greater increases in high amplitude calcium oscillations and greater cell death.

When the authors apply this peptide to primary CLL cells in vitro, they find that calcium oscillations are augmented, and that CLL cells die what appears to be an apoptotic death, because caspases are activated and their nuclei have a characteristic morphology. Furthermore, normal lymphocytes are relatively less affected, suggesting that CLL cells are more dependent on the BCL-2:IP3R interaction than are normal lymphocytes, and providing the possibility of a useful therapeutic window.

However, even though caspases are activated, it is not certain that this represents a traditional BAX- and BAK-dependent apoptotic death. This is important; because it is quite possible that much of the resistance to therapy in CLL is based on increased resistance to apoptosis via the BCL-2 family governed pathway. If this peptide mediates an “end-run” around this control of mitochondrial apoptosis, it might work even in cases of previously poor therapeutic response.

In the case of the BH3 mimetic ABT-737, it is well established that the antiapoptotic...
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