worse left behind, or in normal tissues, remains unclear. Further, the study does not address the issue of heterogeneity within the same sample.

There is also a need to correlate the findings with clinicopathological features and to be able to carry out subset analysis, in particular expanding the analysis of the high-grade/high-p16/high-Ki67 DCIS. This issue also highlights very clearly that a combination of pathological, clinical, and molecular factors may ultimately reveal more powerful and robust measures for disease classification than any one modality alone (Rosai, 2007).

The ability to predict the outcome of an in situ cancer at the time of primary diagnosis would make a huge impact in clinical practice, especially as the frequency of the lesions is rising due to mammographic screening. The authors have set the stage for DCIS, which is generally a segmental disease (Figure 1). Perhaps this will also be the spur to study lobular carcinoma in situ (LCIS), a multifocal proliferation with a bilateral risk of invasive carcinoma, and hence an even bigger dilemma regarding appropriate management.

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NOTCH and PI3K-AKT Pathways Intertwined

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Constitutive signaling by the NOTCH1 receptor contributes to more than half of all cases of T cell acute lymphoblastic leukemia (T-ALL). However, blocking the proteolytic activation of NOTCH1 with γ-secretase inhibitors (GSIs) fails to alter the growth of some T-ALL cell lines carrying the mutated receptor. A recent report by Palomero et al. in *Nature Medicine* identifies loss of PTEN as a critical event leading to resistance to NOTCH inhibition, which causes the transfer of "oncogene addiction" from the NOTCH1 to the PI3K/AKT pathway. This novel observation suggests the need to simultaneously inhibit both pathways as a means to improve therapeutic efficacy in human T-ALL.

NOTCH1 encodes a transmembrane receptor that undergoes a series of activation steps upon ligand binding, culminating in the γ-secretase-mediated proteolytic release of the intracellular fragment of NOTCH1 (ICN). The ICN then translocates to the nucleus, where it is transcriptionally active and required for T cell development (reviewed in Grabher et al., 2006). Aberrant NOTCH1 activation leads to T-ALL in the mouse, and activating mutations occur in more than 50% of cases of human T-ALL (Weng et al., 2004). GSIs, which were initially developed for the treatment of Alzheimer's disease, effectively inhibit the proteolytic activation of NOTCH receptors, a discovery that led to enthusiastic application of NOTCH pathway inhibitors to block cell proliferation and survival in T-ALL. Inhibition of NOTCH1 signaling by GSI treatment proved effective in inducing proliferation arrest or apoptosis in some but not all T-ALL cell lines, suggesting a previously unrecognized mechanism of resistance. In a recent report in *Nature Medicine*, Palomero et al. (2007) show that homozygous loss of *PTEN* is a critical determinant of resistance to GSI-mediated inhibition of NOTCH1 signaling in T-ALL cell lines (Figure 1). They show further that *PTEN* expression is negatively regulated by HES1, a prominent

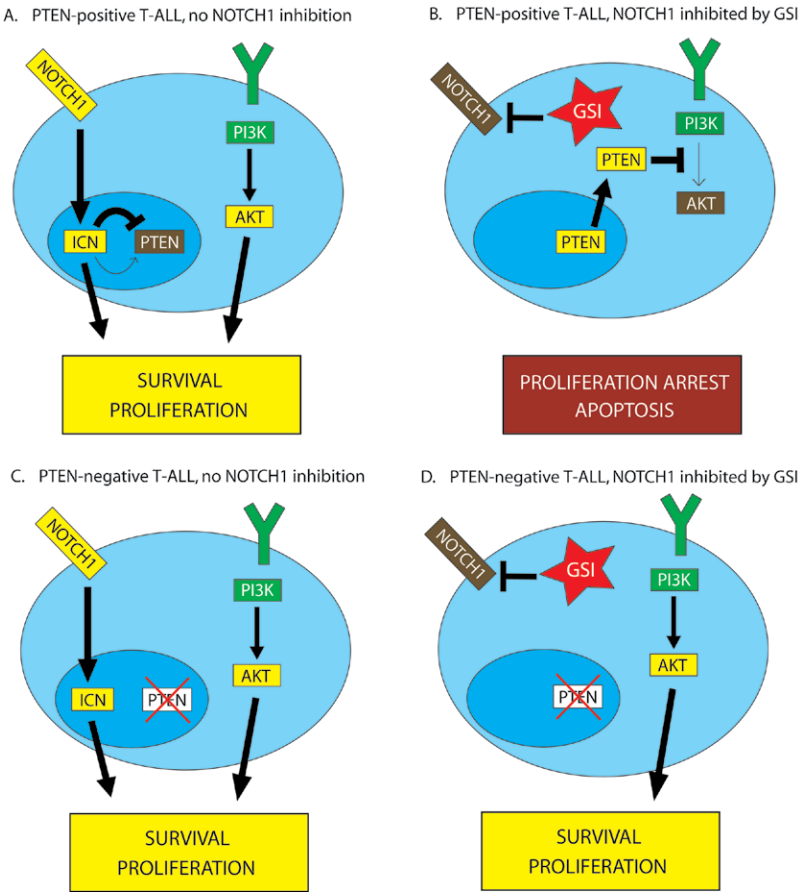

Figure 1. *PTEN* Inactivation Leads to Resistance to NOTCH1 Inhibition but Dependence on AKT Signaling

(A) T-ALL lymphoblasts with activating mutations of *NOTCH1* constitutively generate intracellular NOTCH1 (ICN), whose transcriptional targets include *MYC* and *HES1*. *MYC* is a transcriptional activator of *PTEN*, but *HES1*-mediated repression appears to predominate under ICN signaling conditions. Low expression of *PTEN* leads to incomplete inhibition of the PI3K-AKT pathway.

(B) Inhibition of proteolytic release of ICN from the NOTCH1 receptor by γ-secretase inhibitors (GSIs) blocks ICN-mediated proliferative and survival signals, leading to cell cycle arrest and apoptosis. Additionally, the HES1-mediated repression of *PTEN* expression is relieved, and *PTEN* can therefore inhibit prosurvival signaling mediated by the PI3K-AKT pathway. Thus, *PTEN*-positive T-ALL cells with activating *NOTCH1* mutations depend on NOTCH1 activity for survival and proliferation.

(C and D) In the absence of *PTEN*, uninhibited AKT activation leads to aberrant prosurvival and proliferative signaling independent of NOTCH1 pathway activity, thus leading to resistance to NOTCH1 inhibition. As previewed in this article, Palomero et al. show that *PTEN* null T-ALL cells that are resistant to NOTCH1 inhibition are "addicted" to PI3K/AKT pathway signaling.

downstream target of NOTCH1. Presumably, the NOTCH1-HES1-mediated conditional inhibition of *PTEN* can become constitutive through *PTEN* mutation or deletion, which renders the cells resistant to GSIs by preventing the reactivation of PTEN that typically occurs when NOTCH signaling is blocked. Interestingly, the acquisition of resistance to NOTCH1 inhibition via *PTEN* loss transfers the malignant clone's "oncogene addiction" from the NOTCH pathway downstream to constitutively activated PI3K-AKT signaling, which is normally inhibited by PTEN.

These findings have clear implications for the treatment of T-ALL, as they provide a firm rationale for the use of combination therapy targeting both the NOTCH1 and the PI3K-AKT pathways. A number of inhibitors of various proteins within the PI3K-AKT pathway are currently under clinical development (Hennessy et al., 2005). Although the optimal PI3K-AKT pathway inhibitor, or combination of inhibitors, for the treatment of T-ALL is unknown, the data of Palomero et al. suggest that direct inhibition of AKT is likely to be effective in T-ALL cases harboring *PTEN* mutations. Additionally, the inhibition of mTOR, a downstream effector of AKT signaling, was recently shown to be synergistic with γ-secretase inhibition in T-ALL cell lines, whether or not the lines were sensitive to GSIs alone (Chan et al., 2007). The inhibition of other gene products in the PI3K-AKT pathway in combination with GSI treatment may prove to be similarly synergistic. Finally, a myriad of PI3K-AKT pathway mediators and members of interacting pathways are mutated in human cancer, suggesting the need for additional molecular analysis of T-ALL cases that lack *PTEN* abnormalities (Brugge et al., 2007).

An important question raised by the work of Palomero et al. is whether the NOTCH1 and PI3K-AKT signaling pathways share a common effector that represents the "core" of the oncogenic stimulus in T-ALL. *MYC* comes to mind immediately because of its status as a prominent downstream target of NOTCH1 signaling in human and murine T-ALL (Palomero et al., 2006; Sharma et al., 2006; Weng et al., 2006), the demonstration of addiction to *MYC* in a wide range of experimental tumor models (reviewed in Arvanitis and Felsher, 2005), and the involvement of AKT signaling in *MYC* activation through the inhibition of GSK3β-mediated inactivation of the MYC protein. Thus, MYC could mediate the addiction of leukemic T cells to both NOTCH1 and AKT signaling. Interestingly, two recent reports have documented dominant-negative *FBW7* mutations in many of the same GSI-resistant T-ALL cell lines that were shown by Palomero et al. to harbor *PTEN* mutations (O'Neil et al., 2007; Thompson et al., 2007). Since FBW7 is the recognition component of an E3 ubiquitin ligase that normally targets both MYC and the NOTCH ICN for proteasomal degradation, it is possible that *PTEN* mutations and *FBW7* mutations synergize to render malignant clones independent of NOTCH1 signaling for growth and survival. Further studies are needed to identify

the molecular basis for the selective pressure that exists in T-ALL cells with *NOTCH1* mutations, which results in the outgrowth of malignant clones that harbor inactivating mutations of both *PTEN* and *FBW7*.

One of the most intriguing questions raised by this work is whether interactions between the NOTCH and PI3K/AKT pathways occur in other types of human cancers. If so, the discovery by Palomero et al. and its implications for targeted therapy could have far-reaching consequences.

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