L.E., Wong, M., et al. (2009). Nature *458*, 780–783.

Dong, C., Wu, Y., Yao, J., Wang, Y., Yu, Y., Rychahou, P.G., Evers, B.M., and Zhou, B.P. (2012). J. Clin. Invest. *122*, 1469–1486.

Dong, C., Yuan, T., Wu, Y., Wang, Y., Fan, T.W.M., Miriyala, S., Lin, Y., Yao, J., Shi, J., Kang, T., Lorkiewicz, P., et al. (2013). Cancer Cell *23*, this issue, 316–331.

Kalluri, R., and Weinberg, R.A. (2009). J. Clin. Invest. *119*, 1420–1428.

Lunt, S.Y., and Vander Heiden, M.G. (2011). Annu. Rev. Cell Dev. Biol. *27*, 441–464.

Mani, S.A., Guo, W., Liao, M.J., Eaton, E.N., Ayyanan, A., Zhou, A.Y., Brooks, M., Reinhard, F., Zhang, C.C., Shipitsin, M., et al. (2008). Cell *133*, 704–715.

Sena, L.A., and Chandel, N.S. (2012). Mol. Cell *48*, 158–167.

Selective Blockade of Transport via SERCA Inhibition: The Answer for Oncogenic Forms of Notch?

Ma. Xenia G. Ilagan[1,*] and Raphael Kopan[1,*]
[1]Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63110, USA
*Correspondence: ilaganmg@wustl.edu (M.X.G.I.), kopan@wustl.edu (R.K.)
http://dx.doi.org/10.1016/j.ccr.2013.02.020

NOTCH1, **which is frequently mutated in T cell acute lymphoblastic leukemia, has been an elusive therapeutic target. In this issue of *Cancer Cell*, Roti and colleagues demonstrate that inhibiting SERCA calcium pumps preferentially impairs the maturation of the most common class of oncogenic Notch1 mutants, thus uncovering a potential therapeutic avenue.**

The four mammalian Notch receptors are large type I membrane proteins, sporting an extracellular domain with 29–36 epidermal growth factor (EGF) repeats followed by the conserved Lin12-Notch repeats (LNR) and a heterodimerization domain (HD; Figure 1A). The LNR and HD domains constitute the negative regulatory region (NRR), which maintains the "off" state of the receptor in the absence of ligand. Upon binding of Notch to ligand presented by a neighboring cell, the NRR undergoes a conformational change to expose the S2 site to ADAM metalloprotease cleavage (Figure 1B). This is followed by γ-secretase-mediated cleavage at the S3 site within the transmembrane domain (TMD), which releases the Notch intracellular domain (NICD). NICD translocates to the nucleus, associates with the DNA-binding protein RBPjκ and the transcriptional coactivator Mastermind (MAM/MAML) to activate transcription. Activation is linked to phosphorylation of the PEST domain, its recognition and ubiquitination by the E3 ubiquitin ligase FBW7, and NICD degradation (reviewed in Kopan and Ilagan, 2009).

Because the Notch signaling pathway regulates many fundamental processes during embryonic development and in self-renewing adult tissues, both gain- and loss-of-function mutations in pathway components lead to developmental disorders, cancer, and other adult onset diseases. Best known is the contribution of ligand-independent, activated forms of Notch1 to T-ALL, more than half of which gain activating mutations in the NRR, PEST, or both (Figure 1A; Weng et al., 2004). The mutations in the NRR lead to ligand hypersensitivity and ligand-independent activation, whereas the PEST domain mutations increase the stability of NICD and lead to sustained signaling activity.

The preponderance of *NOTCH1* mutations in T-ALL has fueled the search for effective anti-Notch1 therapeutics (Figure 1B; reviewed in Tzoneva and Ferrando, 2012). Because Notch activation relies on proteolysis, γ-secretase inhibitors (GSIs), which had been originally developed for Alzheimer's disease therapy, have entered clinical trials for treatment of relapsing T-ALL. However, sustained GSI inhibition is not tolerated, because pan-Notch blockade causes severe gastrointestinal toxicity and promotes progression of squamous cell carcinomas (Extance, 2010). The same problems could affect the efficacy of stapled dominant negative MAML-like peptides (SAHM) that directly target the transcription complex. More recently, receptor-specific anti-NRR1 antibodies have been developed. Despite their ability to circumvent gut toxicity, sustained treatment with these reagents will likely cause vascular neoplasms, raising additional safety concerns (Yan et al., 2010).

To identify modulators of Notch1 signaling and potential therapeutic targets for T-ALL, Roti et al. (2013; in this issue of *Cancer Cell*) conducted complementary high throughput small molecule and cDNA overexpression screens using cell-based assays reporting Notch transcriptional activity. For the compound screen, the transcriptional signature of Notch in T-ALL was assembled from previous genome-wide expression profiling studies of multiple human T-ALL cell lines treated with vehicle or GSI. They validated a group of 28 target and 4 nontarget genes to generate a robust,

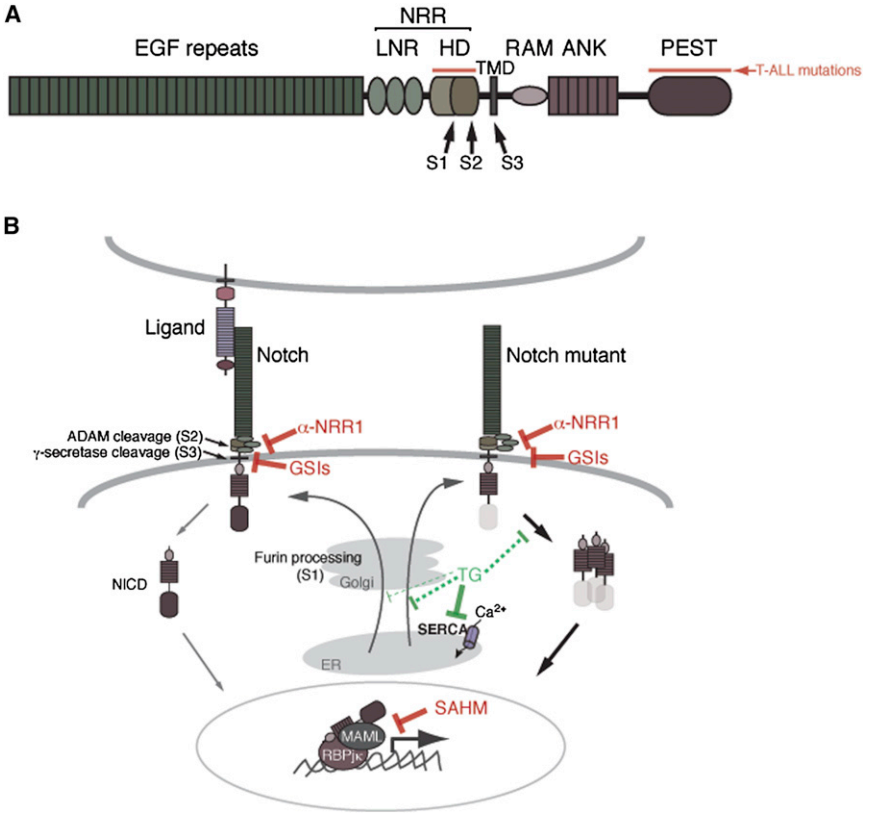

Figure 1. The Core Notch Signaling Pathway and Strategies for Therapeutic Intervention
(A) Domain organization of Notch1 (RAM, RBPjκ-association module; ANK, ankyrin repeats; PEST, proline/glutamic acid/serine/threonine degron domain). Cleavage sites for furin (S1), ADAM metalloprotease (S2), and γ-secretase (S3) are indicated. The NRR keeps the receptor "off" in the absence of ligand. Gain-of-function mutations associated with T-ALL (highlighted in red) predominantly lie within HD, which lead to ligand hypersensitivity and ligand-independent activation, or within PEST, which typically lead to deletions and truncations and therefore increased stability and prolonged signaling activity. HD and PEST activating mutations can also occur in *cis*. Notably, loss-of-function mutations throughout the entire coding region are also associated with cancer, most often in head and neck squamous cell carcinoma. (B) Core Notch signaling pathway. The Notch receptor is processed at cleavage site 1 (S1) by furin-like proteases in the Golgi and is expressed at the cell surface as an intramolecular heterodimer held together via interactions between the N- and C-terminal regions of HD. Upon ligand binding, Notch is sequentially cleaved by ADAM and by γ-secretase, thereby releasing NICD to activate transcription. Notch T-ALL mutants (HD mutant with ΔPEST shown) exhibits ligand-independent receptor activation. Various aspects of the Notch signaling mechanism can be targeted for therapeutic intervention; α-NRR1 antibodies stabilize the auto-inhibited conformation of NRR to prevent S2 cleavage; GSIs prevent cleavage and NICD release, SAHM peptides block transactivation complex function. All these modes of inhibition target wild-type and mutant receptors similarly. SERCA inhibition by thapsigargin (TG) selectively targets the maturation and activity of mutant Notch receptors carrying the most common type of HD mutations found in T-ALL (class I).

highly predictive, and Notch-dependent gene expression signature. Three thousand eight hundred one drugs and drug-like molecules were screened against a human T-ALL cell line (DND41), which carries an activating mutation in the HD of Notch1 along with a PEST domain deletion (L1594PΔPEST). In parallel, they also screened a cDNA expression library of 18,000 open reading frames to identify gene products that would enhance the activation of a transcriptional reporter downstream of another mutant Notch1 receptor identified in T-ALL patients (L1601PΔPEST) expressed in the U2OS osteosarcoma cell line. The selection of NRR mutants in both screens proved to be a fortuitous decision.

Perhaps surprisingly, calcium modulators emerged as hits in both the compound and cDNA screens. One of the top compound hits was thapsigargicin, an analog of thapsigargin, which is a potent natural product inhibitor of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). Among the top cDNA hits were *ATP2A1*, *ATP2A2*, and *ATP2A3*, which encode SERCA1, SERCA2, and SERCA3, respectively. SERCAs use ATP to pump Ca^{2+} from the cytoplasm to the internal stores. Notably, previous studies in *Drosophila* identified the SERCA homolog, Ca-P60A, as a modulator of Notch transport and activity (Periz and Fortini, 1999). However, the loss of all SERCA activity in *Drosophila* demonstrated a general requirement for Ca^{2+}-ATPase for all membrane protein trafficking, which would predict a plethora of untoward effects with SERCA modulation akin to the problems experienced with GSI. This proved not to be the case at low inhibitor concentration.

Because the EGF and LNR domains of Notch all require Ca^{2+} for proper protein folding and exit from ER, Roti et al. (2013) hypothesized that SERCA inhibition was affecting the maturation process of Notch1. Indeed, thapsigargin treatment reduced the level of furin processing, a step that occurs in the trans-Golgi during transport of Notch proteins. Misfolded full-length receptors were retained in the ER/Golgi compartment, leading to diminished levels at the cell surface. Consistent with effects related to the Ca^{2+}-binding modules, constitutively active forms of Notch1 lacking the EGF and LNR domains were refractory to effects of thapsigargin. In addition, expression of NICD can rescue the negative effects of thapsigargin on the cell cycle, cell size, and cell viability of T-ALL lines in vitro and in a xenograft model.

Although the functional assays in flies (Periz and Fortini, 1999; Roti et al., 2013) clearly show that wild-type Notch function can be modulated by SERCA inhibition, the decision to screen against NRR mutants provided a critical observation; the Notch1 mutational status affected the efficacy of thapsigargin. Thapsigargin had a stronger effect on molecules containing the class I HD mutations, which encompass point substitutions and small in-frame insertions or deletions in HD and are the most common type of activating Notch1 mutants in T-ALL (Malecki et al., 2006). At thapsigargin concentrations that did not inhibit signaling from wild-type Notch1 and Notch2 receptors, signaling from the Notch1 receptor carrying the L1601PΔPEST leukemogenic mutation was impaired. Cell cycle and

cell viability were more strongly affected by thapsigargin in T-ALL lines with HD mutations in Notch1 than those carrying wild-type Notch1. Moreover, significant on-target antileukemia effects with no gastrointestinal toxicity were observed in two independent human T-ALL xenograft models carrying HD mutations. The lack of gut toxicity indicates that sufficient levels of wild-type Notch1 and Notch2 receptors reached the surface in the presence of SERCA inhibitors, whereas oncogenic Notch molecules were selectively and effectively prevented from exiting the ER.

Why would SERCA inhibition preferentially affect the maturation and activity of mutant receptors? Roti et al. (2013) speculate that the reason may reflect folding defects in many of the activating HD mutations identified in T-ALL (Malecki et al., 2006). SERCA inhibitors exploit this impaired folding and block maturation of the mutant receptor (Figure 1B). Alternatively or simultaneously, the mutant Notch1 proteins themselves trigger ER stress, making the cells more sensitive to the increase in ER stress induced by thapsigargin treatment, leading to enhanced clearance of mutant Notch proteins. Regardless of the underlying mechanism, these studies provide a therapeutic window for targeting SERCA as an antileukemia strategy for many T-ALL patients harboring mutations in the NRR.

While promising, many challenges remain before translating this strategy to the clinic. Given the fundamental role of calcium in normal physiology and the pleiotropic roles of Notch in tissue maintenance and cancer suppression (South et al., 2012), targeted delivery of SERCA inhibitors to T-ALL cells would be desirable. This was achieved with delivery of modified thapsigargin to human cancer xenografts (Denmeade et al., 2012). Even if thapsigargin can be specifically targeted, T-ALL may contain cells refractory to treatment, having lost the NRR or gained activating Myc mutations. Perhaps the most beneficial use for thapsigargin will be in combinatorial therapies aimed to combat T-ALL at its earliest manifestation before additional mutations are gained.

REFERENCES

Denmeade, S.R., Mhaka, A.M., Rosen, D.M., Brennen, W.N., Dalrymple, S., Dach, I., Olesen, C., Gurel, B., Demarzo, A.M., Wilding, G., et al. (2012). Sci. Transl. Med. *4*, 140ra186.

Extance, A. (2010). Nat. Rev. Drug Discov. *9*, 749–751.

Kopan, R., and Ilagan, M.X. (2009). Cell *137*, 216–233.

Malecki, M.J., Sanchez-Irizarry, C., Mitchell, J.L., Histen, G., Xu, M.L., Aster, J.C., and Blacklow, S.C. (2006). Mol. Cell. Biol. *26*, 4642–4651.

Periz, G., and Fortini, M.E. (1999). EMBO J. *18*, 5983–5993.

Roti, G., Carlton, A., Ross, K.N., Markstein, M., Pajcini, K., Su, A.H., Perrimon, N., Pear, W.S., Kung, A.L., Blacklow, S.C., et al. (2013). Cancer Cell *23*, this issue, 390–405.

South, A.P., Cho, R.J., and Aster, J.C. (2012). Semin. Cell Dev. Biol. *23*, 458–464.

Tzoneva, G., and Ferrando, A.A. (2012). Curr. Top. Microbiol. Immunol. *360*, 163–182.

Weng, A.P., Ferrando, A.A., Lee, W., Morris, J.P., 4th, Silverman, L.B., Sanchez-Irizarry, C., Blacklow, S.C., Look, A.T., and Aster, J.C. (2004). Science *306*, 269–271.

Yan, M., Callahan, C.A., Beyer, J.C., Allamneni, K.P., Zhang, G., Ridgway, J.B., Niessen, K., and Plowman, G.D. (2010). Nature *463*, E6–E7.

Interweaving the Strands: β-Catenin, an HIV Co-Receptor, and Schwann Cell Tumors

David Largaespada[1,2,3,4,5,6,*] and Nancy Ratner[7,*]
[1]Masonic Cancer Center
[2]Department of Genetics, Cell Biology and Development
[3]Center for Genome Engineering
[4]Brain Tumor Program
[5]Health and Natural Sciences Department
[6]Department of Pediatrics
University of Minnesota, Minneapolis, MN 55455, USA
[7]Division of Experimental Hematology and Cancer Biology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH 45229, USA
*Correspondence: larga002@umn.edu (D.L.), nancy.ratner@cchmc.org (N.R.)
http://dx.doi.org/10.1016/j.ccr.2013.03.001

WNT/β-catenin signaling is critical to the development of many cancer types. A paper by Mo and colleagues in a recent issue of *Cell* shows that autocrine CXCL12/CXCR4 chemokine signaling activates β-catenin signaling in a rare peripheral nerve sarcoma. Together with the availability of small molecules targeting CXCR4, this finding suggests new avenues for cancer therapy.

It is exciting to link established signaling pathways. It is especially provocative when compounds designed to target one molecule for a specific disease are shown to have potential in a novel context. In a recent issue of *Cell*, the laboratories of Luis Parada and Lu Le accomplish just this by showing that a pathway that was first identified as relevant to