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.)
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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

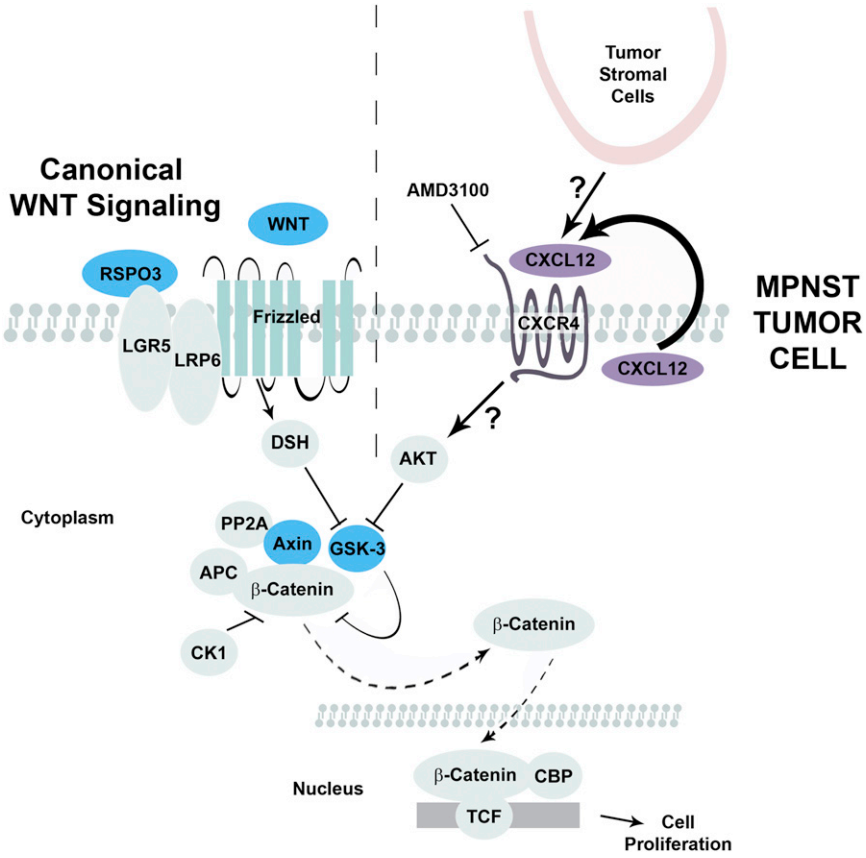

Figure 1. Canonical WNT Signaling and CXCR4 Activation of β-Catenin
In canonical WNT signaling (left of dotted line), WNT ligands activate FRIZZLED receptors; LRP and LGR are co-receptors. Receptor activation leads through inactivation of GSK3β to stabilization of β-catenin. Stabilized β-catenin moves to the nucleus and activates gene transcription. Mo et al. (2013) (right of dotted line) describe a novel mechanism in which MPNST tumor cells secrete CXCL12 ligand, activate CXCR4 receptors, and, via AKT, inactivate GSK3β and stabilizing β-catenin.

lymphocyte chemotaxis is also a driver of human Schwann cell tumor progression (Mo et al., 2013). The authors go on to find that this pathway acts via another pathway that was initially identified in organismal development and found to be corrupted in many cancers. The paper links the CXCR4 cell surface chemokine receptor, via autocrine CXCL12 ligand production, to the Wnt/β-catenin signaling pathway in malignant peripheral nerve sheath tumors (MPNSTs) (Mo et al., 2013). CXCR4 antagonists are being intensively investigated, because CXCR4 is a co-receptor for HIV on T cells, and early stage clinical studies show that blocking CXCR4 delays the onset of AIDS in HIV infected individuals (Domanska et al., 2013). The work by Mo et al. (2013) suggests that CXCR4 antagonists may be useful to treat MPNSTs, a peripheral nerve related soft tissue sarcoma with very poor prognosis, especially when they occur in the context of neurofibromatosis type 1 (NF1) disease (Widemann, 2009). In MPNST, inactivation of the *NF1* gene, which encodes a GTPase activating protein for Ras proteins, increases Ras signaling with activation of the key downstream signaling pathways MEK, AKT, and mTOR (De Raedt et al., 2011; Jessen et al., 2013). The paper by Mo et al. (2013) also shows expression of CXCR4 and Wnt/β-catenin pathway components in benign neurofibromas, which can be MPNST precursor lesions.

The WNT/β-catenin signaling pathway was identified for its roles in development and controls critical processes in worms to mammals, from the formation of teeth to the control of stem cells in the intestine (reviewed in Clevers and Nusse, 2012). Despite >7,000 papers on WNT/β-catenin signaling listed in the PubMed database, this pathway had not been directly implicated in MPNSTs. In the cells from which MPNSTs derive, which may be neural crest cells, skin-derived precursors, and/or committed Schwann cells, WNT/β-catenin signaling normally regulates cell fate decisions and transiently suppresses full differentiation (myelination) in Schwann cells, potentially explaining a role in tumor progression by differentiation block (Lewallen et al., 2011; Hari et al., 2012).

Canonical WNT/β-catenin signaling (Figure 1) plays a role in many types of cancer, including colorectal, lung, breast, ovarian, prostate, liver, and brain tumors (Clevers and Nusse, 2012; Saito-Diaz et al., 2013). β-catenin-dependent transcription can promote cell cycle progression, stem cell self-renewal, and epithelial-to-mesenchymal transition. WNT signaling in cancer can be aberrantly activated by activation of mutations in β-catenin (*CTNNB1*), overexpression of WNT ligand genes, inactivation of mutations in destruction complex genes (i.e., *AXIN1*, *GSK3B*, and *APC*), or promoter hypermethylation of negative regulators of WNT signaling (Saito-Diaz et al., 2013). Activation of the PI3K/AKT signaling pathway, either by the loss of *PTEN* or through activation of upstream tyrosine kinase receptors, also causes phosphorylation and inactivation of GSK3β, stabilizing β-catenin (Clevers and Nusse, 2012). In the study by Mo et al. (2013), WNT signaling is activated by crosstalk with the CXCL12/CXCR4 signaling, downstream of AKT (Figure 1).

CXCL12 (SDF-1) is a chemokine. CXCL12 binding to the heterotrimeric G protein-coupled receptor CXCR4 is, like β-catenin signaling, required for normal development. In many situations, stromal cells secrete CXCL12 and attract cells expressing CXCR4 receptors. In this fashion, immune cells are attracted to sites of inflammation and hematopoietic cells home to the bone marrow. This paracrine CXCL12/CXCR4 pathway has been exploited therapeutically; CXCR4 blockade facilitates the removal of cells from the bone marrow niche for use in transplantation (reviewed in Domanska et al., 2013). CXCL12/CXCR4 paracrine signaling is also relevant to tumor metastasis when tumor cells expressing CXCR4 migrate toward distant sites where ligand is produced (Domanska et al., 2013).

The paper by Mo et al. (2013) shows that autocrine, rather than paracrine, CXCL12 promotes tumor cell proliferation. The notion that tumor cells can manufacture, secrete, and respond to their own CXCL12 was discovered in prostate tumors by Sun et al. (2003). Mo et al. (2013) use antibodies against CXCL12 and CXCR4 receptor blockade, both of which decrease MPNST cell proliferation. Furthermore, whereas CXCR4 activation can recruit endothelial cells to promote neoangiogenesis, vessel density remains the same after CXCR4 blockade, providing additional evidence that the tumor cell effects are cell autonomous. CXCL12 produced in a paracrine manner by host tumor stromal cells, as in other forms of cancer, may also contribute to effects on tumor growth.

The authors show that CXCR4 activation in MPNST cells activates β-catenin by AKT-mediated phosphorylation and inactivation of GSK3β, thus stabilizing β-catenin (Figure 1). How CXCR4 activates AKT is not entirely clear. This may occur through the βγ subunits of CXCR4, known to indirectly activate AKT (Domanska et al., 2013). The authors exclude roles for activation of NF-κB, RAS/MAPK, and JAK/STAT3. Additional pathways downstream of CXCR4 might also contribute to β-catenin stabilization. CXCR4 is known to activate SRC family kinases, C-CBL, and RHO GTPases (Domanska et al., 2013), which may be relevant. β-catenin activation likely also requires additional genetic events or the activation of signaling pathways in neurofibromas and MPNSTs, particularly in MPNSTs that develop in the absence of NF1 syndrome. This is because many MPNSTs express β-catenin, but do not express CXCR4. Also, many neurofibromas express CXCR4, but not β-catenin.

The molecular mechanisms that explain CXCL12 and CXCR4 expression in MPNST cells also remain undefined. One possibility is that CXCR4/CXCL12 expression reflects the embryonic neural crest origin of MPNST cells. The authors demonstrate that CXCR4 expression frequency and intensity are especially pronounced in neurofibromas and MPNSTs associated with NF1 disease. Therefore, signaling downstream of NF1 may normally suppress expression of CXCL12/CXCR4.

Despite many efforts to identify drugs to target β-catenin signaling, no inhibitor has, to date, demonstrated the appropriate pharmacodynamic and pharmacokinetic properties to be used as a drug for the treatment of cancer patients. In this light, it is impressive that the authors have shown that the CXCR4 inhibitor AMD3100 inhibits β-catenin signaling in vitro and in vivo. With AMD3100 (Plerixafor; FDA approved for use in hematopoietic stem cell mobilization) currently in clinical testing in many settings, it should be feasible to test in human MPNST patients. Mo et al. (2013) show a clear delay in MPNST formation in human xenografts and also in a genetically engineered mouse model. Certainly, targeting the CXCR4 receptor alone is insufficient to halt MPNST growth or shrink tumors; the authors show that the main effect is proliferation arrest. Moreover, the authors note that some tumors expressed CXCR4 in a patchy manner, where the cells are not all positive. For both of these reasons, tumor ablation is likely to require co-therapy, perhaps with identified NF1/Ras pathway inhibitors (De Raedt et al., 2011; Jessen et al., 2013). Especially because *NF1* mutations have been identified in many types of cancer, it will be exciting to discover those that use the CXCL12/CXCR4 pathway to activate Wnt/β-catenin signaling and those that may benefit from CXCR4 blockade.

REFERENCES

Clevers, H., and Nusse, R. (2012). Cell *149*, 1192–1205.

De Raedt, T., Walton, Z., Yecies, J.L., Li, D., Chen, Y., Malone, C.F., Maertens, O., Jeong, S.M., Bronson, R.T., Lebleu, V., et al. (2011). Cancer Cell *20*, 400–413.

Domanska, U.M., Kruizinga, R.C., Nagengast, W.B., Timmer-Bosscha, H., Huls, G., de Vries, E.G., and Walenkamp, A.M. (2013). Eur. J. Cancer *49*, 219–230.

Hari, L., Miescher, I., Shakhova, O., Suter, U., Chin, L., Taketo, M., Richardson, W.D., Kessaris, N., and Sommer, L. (2012). Development *139*, 2107–2117.

Jessen, W.J., Miller, S.J., Jousma, E., Wu, J., Rizvi, T.A., Brundage, M.E., Eaves, D., Widemann, B., Kim, M.O., Dombi, E., et al. (2013). J. Clin. Invest. *123*, 340–347.

Lewallen, K.A., Shen, Y.A., De la Torre, A.R., Ng, B.K., Meijer, D., and Chan, J.R. (2011). J. Neurosci. *31*, 3032–3043.

Mo, W., Chen, J., Patel, A., Zhang, L., Chau, V., Li, Y., Cho, W., Lim, K., Xu, J., Lazar, A.J., et al. (2013). Cell *152*, 1077–1090.

Saito-Diaz, K., Chen, T.W., Wang, X., Thorne, C.A., Wallace, H.A., Page-McCaw, A., and Lee, E. (2013). Growth Factors *31*, 1–31.

Sun, Y.X., Wang, J., Shelburne, C.E., Lopatin, D.E., Chinnaiyan, A.M., Rubin, M.A., Pienta, K.J., and Taichman, R.S. (2003). J. Cell. Biochem. *89*, 462–473.

Widemann, B.C. (2009). Curr. Oncol. Rep. *11*, 322–328.