Thus, whereas loss of HIF-1 or HIF-2 activity in ECs is compatible with normal cardiovascular development, the combined loss of HIF-1 and HIF-2 activity is not. Overexpression of HIFdn may have confounding effects, such as competitive binding to proteins that interact with HIF-2α at residues not deleted in HIFdn, resulting in their cytoplasmic sequestration. It will be interesting to determine whether EC-specific HIF-1β deficiency phenocopies HIFdn and whether combined loss of HIF-1 and HIF-2 at earlier developmental stages (ie, prior to *Flk1* expression) reveals essential roles for these proteins in hemangiopoiesis or vasculogenesis. Nevertheless, the results of Licht et al provide strong evidence that HIF-1 and HIF-2 play critical intrinsic roles in ECs. Because of their dual (extrinsic and intrinsic) effects, targeting these factors may represent a powerful approach to inhibiting angiogenesis in cancer and other disorders that are dependent upon neovascularization. ■

REFERENCES

1. Semenza GL. Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology (Bethesda). 2004;19:176-182.

2. Raval RR, Lau KW, Tran MG, et al. Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. Mol Cell Biol. 2005;25:5675-5686.

3. Kaelin WG Jr. The von Hippel-Lindau protein, HIF hydroxylation, and oxygen sensing. Biochem Biophys Res Commun. 2005;338:627-638.

4. Manalo DJ, Rowan A, Lavoie T, et al. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. Blood. 2005;105:659-669.

5. Tang N, Wang L, Esko J, et al. Loss of HIF-1α in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. Cancer Cell. 2004;6:485-495.

6. Scortegagna M, Ding K, Oktay Y, et al. Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1−/− mice. Nat Genet. 2003;35:331-340.

● ● ● **IMMUNOBIOLOGY** ◗

Comment on Bilancio et al, page 642

Knock-outs and inhibitors: one and the same?

Zachary A. Knight and Kevan M. Shokat UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

A comparative analysis of isoform-specific PI3K inhibitors and cells from kinase-dead knock-in mice confirms a key role for p110δ in B-cell signaling.

Phosphoinositide 3-kinases (PI3Ks) generate lipid second messengers that are critical for diverse aspects of the immune response. Two PI3Ks (p110γ and p110δ) are preferentially expressed in leukocytes, and knock-out (KO) mice for these 2 kinases reveal unique defects in immune signaling. p110γ KO animals exhibit impaired neutrophil and macrophage chemotaxis,[1,2] whereas mice lacking p110δ show defective signaling from the B- and T-cell antigen receptors,[3,4] among other phenotypes. Based on these data, the pharmaceutical industry has aggressively pursued selective inhibitors of these enzymes as potential drugs for the treatment of autoimmune disease.

Unfortunately, the story is not quite so simple. The same studies that implicated PI3Ks in diverse immune signaling also raised questions about what a drug targeting these kinases would really do.[5] One strain of p110γ KO mice, but not others, is prone to colon cancer, and the molecular basis for this difference is unknown.[6] p110γ KO mice suffer from increased cardiac contractility, whereas those that express a kinase-dead (KD) p110γ (which better mimics the effects of an inhibitor) do not.[7] Conversely, animals expressing p110δ KD exhibit more pronounced defects in lymphocyte signaling and development than p110δ KO mice.[8] Taken together, these data have served to remind the scientific community that subtly different types of knock-outs can induce very different phenotypes, and that none of these necessarily anticipate the effect of a small molecule drug (see figure).

In this issue of *Blood*, Bilancio and colleagues directly address this question in murine B cells by comparing pharmacologic and genetic inactivation of PI3K isoforms. The authors use the first selective inhibitors of p110γ and p110δ to define the role of these 2 kinases in signaling from the B-cell antigen and IL-4 receptors, and then compare these results with B cells from p110δ KD mice. They report that the p110δ inhibitor IC87114 potently inhibits B-cell receptor–induced proliferation, calcium mobilization, and activation of downstream PI3K effectors such as Akt—all of which are recapitulated by cells from p110δ KD mice. By contrast, the p110γ inhibitor AS-604850 has no effect on these signaling events, excluding an essential role for p110γ in signaling from the B-cell receptor. Because small molecule inhibitors always possess imperfect specificity, the authors' use of 2 compounds with complementary selectivity helps to reinforce these conclusions.

One intriguing observation from this study is that the p110δ inhibitor IC87114 is significantly more potent in B cells ($IC_{50} = 0.04$–$0.14\ \mu M$) than in most other cells (typical $IC_{50} = 1$-$5\ \mu M$). This presumably reflects the fact that B-cell signaling is tuned to be more sensitive to the amount of p110δ activity than signaling in other cell types (including other leukocytes that highly express p110δ) and suggests that in the intact organism, B cells are likely to be more sensitive to pharmacologic disruption by p110δ inhibitors. As PI3K isoforms often collaborate to synthesize the same lipids within the same cell, defining these isoform and cell-type–specific thresholds represents a key challenge for understanding signaling by this family of enzymes. Pharmacologic approaches such as the one reported here by
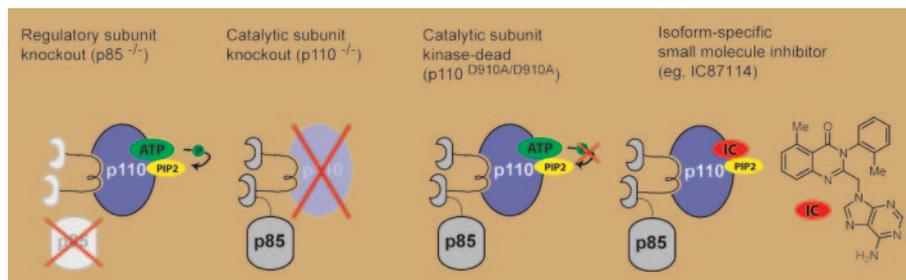
Genetic and pharmacologic approaches for inactivating the class IA PI3Ks, which are heterodimers of catalytic (p110) and regulatory (p85) subunits.

Bilancio and colleagues will be an important tool for meeting this challenge. ■

REFERENCES

1. Li Z, Jiang H, Xie W, Zhang Z, Smrcka AV, Wu D. Roles of PLC-beta2 and –beta3 and PI3Kgamma in chemoattractant-mediated signal transduction. Science. 2000;287:1046-1049.

2. Hirsch E, Katanaev VL, Garlanda C, et al. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. Science. 2000;287:1049-1053.

3. Okkenhaug K, Bilancio A, Farjot G, et al. Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. Science. 2002;297:1031-1034.

4. Jou ST, Carpino N, Takahashi Y, et al. Essential, nonredundant role for the phosphoinositide 3-kinase p110delta in signaling by the B-cell receptor complex. Mol Cell Biol. 2002;22:8580-8591.

5. Vanhaesebroeck B, Rohn JL, Waterfield MD. Gene targeting: attention to detail. Cell. 2004;118:274-276.

6. Sasaki T, Irie-Sasaki J, Horie Y, et al. Colorectal carcinomas in mice lacking the catalytic subunit of PI(3)Kgamma. Nature. 2000;406:897-902.

7. Patrucco E, Notte A, Barberis L, et al. PI3Kgamma modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects. Cell. 2004;118:375-387.

8. Okkenhaug K, Vanhaesebroeck B. PI3K-signalling in B- and T-cells: insights from gene-targeted mice. Biochem Soc Trans. 2003;31:270-274.

● ● ● ● NEOPLASIA

Comment on Holleman et al, page 769

Bcl-rambo: a maverick of apoptotic genes

James Feusner CHILDREN'S HOSPITAL AND RESEARCH CENTER OF OAKLAND

Holleman and colleagues report finding differential expression of groups of apoptotic genes with differing subgroups of ALL, but only one such gene associated significantly with drug resistance and risk of relapse—BCL2L13 (Bcl-rambo).

Apoptosis, mitochondrial or death-receptor mediated, is one of the main mechanisms of tumor cell death induced by chemotherapeutic agents. Cellular drug resistance has been linked with unfavorable prognosis in leukemia,[1] and with a decreased ability to induce apoptosis. To date, few genes have been examined as potential mediators of drug resistance through apoptosis. In this issue of the journal, Holleman and colleagues report the results of their extensive evaluation of 70 apoptosis genes in this regard.

The authors studied children treated both in Holland and in the United States (St Jude) for acute lymphoblastic leukemia (ALL). This work is the first to describe an association between differential apoptotic gene expression and leukemia lineage, genetic subtype, and in vitro drug resistance in childhood ALL. Although only one of these genes was found to associate independently with patient outcome, it is of considerable interest.

This gene, BCL2L13, was also found to be significantly related to resistance to one drug, L-asparaginase. The clinical significance of this is the potential for down-regulation of this gene by antisense oligonucleotides or specific inhibitor therapy. This approach could sensitize the ALL cells to L-asparaginase.

This is exciting news, but must be tempered a bit. First, the number of patients studied was relatively small (190 from Holland, 92 from St Jude). Second, this was a retrospective study and would need to be validated in a prospective manner. Third, the authors did not have enough patients with T-lineage ALL to study it meaningfully. This is unfortunate, since this is a large subgroup of pediatric ALL and is one of the most difficult to treat. And last, some of the analyses for risk of relapse involved very small patient numbers (eg, < 10 each for BCR-ABL, E2A-rearranged, or MLL-rearranged cases).

Of interest, the association of this gene's expression and outcome was counterintuitive. Increased messenger RNA levels of this gene were associated with cellular resistance to L-asparaginase and an increase relapse rate. Based on cell line work, one would have expected an increased expression of this proapoptotic gene to have led to an increase in cell kill and hence fewer relapses. One explanation offered by the authors is posttranslational production of splice variants that have relatively more antiapoptotic function. Protein expression and function studies will need to be conducted to sort this out.

We look forward to others duplicating this work, in prospective studies. If the finding of Holleman et al is confirmed, it could lead to significant clinical improvement in outcome, since L-asparaginase is very important to long-term outcome for many children with ALL.[2,3] ■

REFERENCES

1. Hongo T, Yajima S, Sakurai, et al. In vitro drug sensitivity testing can predict induction failure and early response of childhood acute lymphoblastic leukemia. Blood. 1997;89:2959-2965.

2. Asselin BL, Dreissman S, Coppola DJ, et al. Prognostic significance of early response to a single dose of asparaginase in childhood acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 1999;21:6-12.

3. Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97:1211-1218.