mutants (I800M and I800L). In addition, the yeast assay identified mutations that convey enhanced sensitivity to inhibitors; one example is L814C. The mutation I800L is unique in that it induces a split phenotype: resistance against some inhibitors and enhanced sensitivity against others. The results obtained with yeast were validated in mammalian systems: the mutation-induced changes in catalytic and signaling activities and in resistance or sensitivity to inhibitors could be faithfully reproduced in human cells, in which the catalytically active mutants retained oncogenic potential.

We can derive several lessons from this study. The resistance mutations identified in the affinity pocket can guide a preemptive strike. It is probably not too early to start generating small-molecule inhibitors that are effective against the I800L and I800M mutants. The L814C mutant, showing increased sensitivity to inhibitors, is a potentially useful tool for the study of isoform-specific functions of p110α. There are currently no isoform-specific inhibitors available for p110α. Therefore, cells carrying a knockin-sensitizing mutation could be used with available compounds at low enough inhibitor concentrations to analyze the selective effects on p110α.

Despite the general structural similarities between kinases, there are sharp differences between protein and lipid kinases. A reflection of these differences is the pronounced intolerance of the PI3K affinity pocket to mutation. A functional explanation of this remarkable inflexibility remains an important goal for future research.

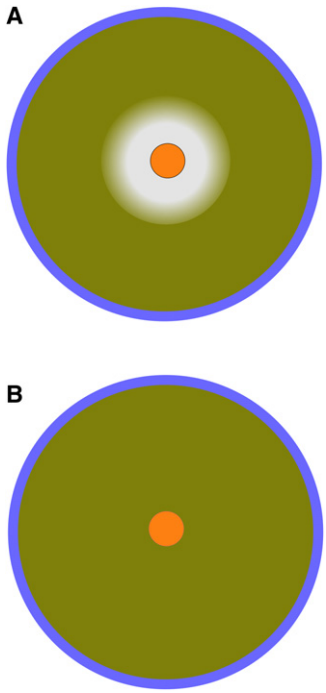

Figure 1. Testing for PI3K Inhibitors in Yeast: The "Reverse Halo" Assay
The two plates contain a lawn of yeast cells that express p110α and hence fail to grow. A PI3K inhibitor spotted on a cellulose disk (orange) diffuses into the surrounding lawn, inhibits p110α, and restores cell growth (A). A control disk with DMSO has no effect (B).

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The Ever-Lengthening Arm of p53

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p53 is a pivotal bulwark against cancer, but exactly how it suppresses tumors remains elusive, in part because it modulates such diverse biological processes via so many downstream pathways. In a recent issue of *Cell*, Godar et al. (2008) now identify another string to p53's anticancer bow—repression of the CD44 cell-surface glycoproteins that coordinate many attributes of tumor progression.

As principal cell factotum of stress responses, sentinel of damage, guardian of the genome, and scourge of all cancers, the p53 protein has assumed an almost mythological status. Such remarkable attributes have garnered p53 much attention from the scientific community, and, some 46,581 scientific publications later (as of July 21, 2008), one might be forgiven for thinking that we know all there is to know about this eldritch protein. Well, actually, no. There are just a few questions that remain

unanswered. Let's start with, "How does p53 suppress cancer?" And when that's done, what about why, where, and when?

The problem is that while we know a lot of potentially tumor-suppressive things that p53 can do—like growth arrest, repair, autophagy, and apoptosis—we know embarrassingly little about which of these effector programs is actually responsible for quelling cancer in any specific instance. Likewise, many signals can activate p53—DNA damage and chromatin disruption, oncogene activation, physical injury, hypoxia, and misfolded proteins, to name but a few—but we have little idea which of them is responsible for engaging p53 during the evolution of any specific tumor type, or when during the protracted process of tumor evolution such engagement occurs. Does p53 sense some universal hallmark of tumorigenicity (perhaps chronic DNA damage or aberrant Myc or E2F activity), or is it instead activated by many different triggers that, between them, encompass the gamut of aberrations in every different type of tumor? Perhaps all that we can be sure of, given the dire consequences of untimely p53 activation, is that whatever triggers p53 in cancers is peculiar to tumor, and not normal, cells.

Transcription factors like p53 make versatile hooks on which evolution can hang mechanistically diverse processes that, when coactivated, coordinate complex biological programs like cell proliferation, differentiation, and tumor suppression. Moreover, evolution can refine, elaborate, or retask such programs by the simple expedient of appending requisite promoter/enhancer recognition elements to new gene targets, and it is the nature of such programs to evolve ever more complexity over time. In this context, it is worth noting that it is very unlikely that primeval p53 started out as a tumor suppressor. p53 is an evolutionarily ancient animal invention (Nedelcu and Tan, 2007) already ensconced in the earliest metazoans, which, since they were typically small and short-lived with postmitotic bodies, had no need for quelling renegade somatic cells. Probably, p53 initially evolved as a transcriptional coordinator of cellular stress and damage responses, ensuring consistent development and integrity of the germline despite the vicissitudes of a capricious environment. Tumor suppression is only necessary in large, long-lived organisms like vertebrates whose component cells engage in continuous renewal, accumulating somatic mutations in the process. p53's role in preventing cancer appears to be a highly specialized and (some might say, given the high incidence of human cancer) rather shoddy retasking of p53's primordial stress and checkpoint functions. This makeshift evolutionary legacy is plainly evident in the multiple, sometimes conflicting, roles played by mammalian p53. The same p53 that activates repair and autophagy to ensure survival and recovery of cells exposed to repairable damage, transient stress, or nutrient privation also activates irreversible arrest and/or death in cells that are severely or persistently injured. Routing two diametrically opposing programs through the same effector is redolent of that unique brand of unintelligent design so favored by evolution. Matters are further compounded by the fact that basal p53 (i.e., p53 in the absence of overt activating signals) regulates key aspects of cell metabolism (Bensaad and Vousden, 2007; Corcoran et al., 2006), development (Stiewe, 2007), reproduction (Hu et al., 2008), and stem cell renewal (Gatza et al., 2007)—all normal physiological processes. Such multitasking of p53 inevitably entails some suboptimal compromises. While p53 does a reasonable job of protecting us from cancer, it is also largely responsible for the devastating side effects of chemotherapy and radiation exposure (Christophorou et al., 2006) and perhaps, after a lifetime of dutifully culling damaged stem cells, the depredations of aging (Gatza et al., 2007).

Because p53 is involved in so many different processes, not all p53-activated genes will be engaged in tumor suppression, and not all p53-suppressed genes will be oncogenic. How, then, to parse which of p53's target genes specifically serve tumor suppression? Into this fractious fray jump Godar et al. (2008). Their discovery that p53 negatively regulates the protean CD44 cell-surface glycoproteins is highly provocative and might indicate a key role for CD44 in tumorigenesis. But equally, it might reflect a role for CD44 in any of the other things that p53 does—DNA damage response, metabolism, development, stem cell renewal, or a combination of some or all of the above.

CD44 is the antigenic moniker for a protean ensemble of cell-surface transmembrane glycoproteins, all generated by alternative splicing of ten central exons transcribed from a single, highly conserved gene (Ponta et al., 2003). Whereas the shortest CD44 isoform is expressed ubiquitously, the others are variously restricted to different tissues and/or stem cell compartments. Provocatively, expression of CD44 (usually its larger isoforms) is frequently elevated in epithelial and some hematopoietic tumors, most notably in those cell subpopulations that comprise tumor-initiating cells (Naor et al., 2008). However, the overexpression of CD44 in cancer cells is not due to mutations in the *CD44* gene but instead appears to be due to its induction by inflammatory cytokines like IL-1β and osteopontin and by oncogenic signals such as β-catenin/Tcf-4 and Ras-Raf-ERK pathways. Ras-Raf-ERK signaling also modulates CD44 alternative splicing. Quite how CD44 contributes to malignancy is unclear, because CD44 proteins participate in a bewildering diversity of biological processes. They act as receptors for extracellular matrix components such as hyaluronic acid, collagen, and fibronectin and interface between epithelial and endothelial cells through interactions with L- and E-selectins. They also modulate availability of growth-factor receptors by recruiting matrix metalloproteases to the extracellular surface and serve as obligate coreceptors for members of the EGFR and Met receptor tyrosine kinase families. The intracellular CD44 effector domain is similarly multifaceted, interacting directly with a subset of cytoskeletal components and various intracellular signaling molecules—most notably the guanine nucleotide exchange factors p115RhoGEF and LARG, through which CD44 activates the RhoA GTPase pathway, and TIAM1 and Vav2, which engage the Rho GTPases Rac1 and Cdc42. Between them, RhoA, Rac1, and Cdc42 are likely responsible for most of the protumorigenic properties of CD44, driving cell growth and survival, promoting tumor cell migration and invasion, and facilitating adhesion of metastatic tumor colonies to distal stroma and endothelium (Bourguignon, 2008).

Godar et al. (2008) start from the simple correlative observation that CD44 expression is high in p53-negative mammary epithelial cells and demonstrate that p53 can directly suppress CD44 via a p53-responsive element in the *CD44*

promoter. But what does this have to do with p53-mediated tumor suppression? More compelling in this regard is their evidence that CD44 overexpression antagonizes the tumor-suppressive apoptotic and growth-inhibitory actions of p53 in mammary epithelial cells and that tumorigenicity of transformed mammary epithelial cells requires *CD44* derepression. Taken together, these data strongly support a critical role for *CD44* in tumorigenesis and the importance for tumor suppression of its repression by p53. However, this is unlikely to be the whole story. Intriguingly, Godar et al. (2008) also offer evidence that CD44 expression is regulated by basal p53. This intimates that the regulation of CD44 by p53 also plays a part in normal physiological functions. Given the role that CD44 appears to play in various hematopoietic and epithelial stem cell compartments, this could be one of the emerging links between p53 and stem cell self-renewal.

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