derivatives of the lead compound they named diminutol, a purine analog that destabilized tubulin in the cell-free extracts and in cells. Active and inactive analogs were covalently attached to agarose beads for affinity-based target identification experiments. Although affinity-matrix based approaches have been used to identify protein targets of potent natural products (e.g., FK506 and myriocin, with activities on cells at nanomolar or lower concentrations [17, 18]) and also bioactive purine derivatives (e.g., purvalanol, cell growth inhibition, and GI_{50} 2.5 μM [19]), a noteworthy aspect of the report by Wignall et al. is the use of this strategy to identify the target of a less-potent compound. In cellular contexts, diminutol is active at 50 μM but is not very active at 10 μM. Using state-of-the-art mass spectrosocopy, the authors were able to identify the target protein from rather complex mixtures of proteins that selectively bind the affinity matrices. These data bode well for other phenotype-based screens that identify small molecules with interesting biological activities, often with activities in the micromolar range, but whose molecular targets are not readily apparent through phenotypic analysis or functional assays.

A surprising result of this study is that one target of diminuntol is NQO1, an NADP-dependent oxidoreducase that can catalyze the two-electron reduction of quinone compounds and is known to be overexpressed in certain tumors (reviewed in [20]). NQO1 has not previously been linked to regulation of microtubule dynamics, in dividing or nondividing cells. This finding presents an exciting opportunity for further research to examine how this enzymatic pathway regulates microtubule function in different cellular contexts. Dimuntol will no doubt be a valuable tool for these studies.

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Targeting FOXO Kills Two Birds with One Stone

PTEN deficiency activates Akt signaling and results in a variety of human malignancies. Encouragingly, recent studies demonstrate that small molecules can regulate FOXO1a, an Akt target, to suppress tumor growth, and FOXO1a is therefore a promising anticancer drug target.

Molecular targeting of tumor-specific signal transduction pathways is a promising strategy for discovering and developing novel potent anticancer drugs. Small-molecule libraries have been intensively screened for compounds that block ligand-receptor interaction [1], those that block signaling transduction pathways [2], and those that block master regulators of cell cycle arrest or apoptosis [3]. Among many possible targets, there is little doubt that phospho-Akt has a critical role in many human cancers, often as a consequence of PTEN inactivation [4]. PTEN, both a lipid phosphatase and a protein phosphatase, is frequently mutated in a wide range of human malignancies, including glioma, prostate and breast tumors, melanoma, squamous cell carcinoma, and thyroid tumors [5, 6]. Animal models have shown that PTEN knockout is embryonic lethal and that heterozygous PTEN-depleted mice are prone to the development of various cancers [7, 8]. Restoring PTEN function in mutant PTEN-containing tumor cells by expressing exogenous PTEN can largely reverse the malignant phenotype. The main mechanism of cancer development due to PTEN inactivation is constitutive activation of Akt function. Akt is a kinase that has numerous targets, many of which are important for regulating the balance between cell survival and apoptosis [9, 10]; such targets include MDM2, Bad, Bcl-2, CDK inhibitors, caspase 9, and forkhead transcription factors such as

FOXO1a, FOXO3a, and FOXO4. Phosphorylation of these targets by Akt activates or reduces their function in favor of cell growth. Notably, Akt-mediated forkhead phosphorylation leads to cytoplasmic retention and loss of activation of transcription of pro-apoptotic genes such as Fas ligand [11] and cell cycle modulators [12, 13]. Currently, there is no specific anti-Akt drug in the clinic or in clinical trials, but there are preclinical data implicating phospho-Akt as a prognostic marker and as an important pathogenic event in cancer [14]. Furthermore, there is a growing literature about the underlying mechanisms of Akt action with respect to cell survival [4, 5, 10].

Based on the fact that Akt plays an important role in tumor development, especially when PTEN is inactivated and phosphorylation by Akt leads to FOXO1 nuclear export, Kau et al [15] performed a cell-based, high-throughput, genetic screen of three different libraries of small molecules to look for effective inhibitors of the PI3K/PTEN/Akt pathway, which may, at least in part, mimic PTEN function. For this analysis they used a PTEN-null cell line that expresses exogenous FOXO1a by adenovirus infection and treated the cells with compounds from the libraries. Exogenous FOXO1a accumulated in the cytoplasm as a result of phosphorylation by constutively active Akt in the PTEN-null cells. As positive controls, they employed widely used PI3K inhibitors to suppress Akt function and the nuclear export inhibitor LMB to induce translocation of cytoplasmic FOXO1a to the nucleus. They screened more than 18,000 compounds from the NCI structural diversity set, ChemBridge DiverSetE, and a small collection of NCI marine extracts in an attempt to identify compounds that relocalize FOXO1a to the nucleus in the PTEN-null cells. By directly visualizing the subcellular localization of FOXO1a, they successfully identified 42 compounds with the ability to direct FOXO1a to the nucleus.

Because phosphorylation and subsequent localization of FOXO1a is a downstream event in the PI3K/PTEN/Akt pathway and reconstitution of forkhead activity is sufficient to restore the induction of apoptosis in PTEN-null cells, any step of the pathway blocked by the compounds would show the same phenomenon. The authors classified their compounds that translocate FOXO1a to the nucleus into two categories. (1) General nuclear-export inhibitors that target the general nuclear-export machinery. Among the 42 compounds identified, 19 compounds fell into this category, and these all affect the nuclear export mediator, CRM1 [16]. (2) Compounds that are not in the first category and that target the PI3K/Akt/FOXO1a pathway. In the second category, the compounds were further classified according to their target in the PI3K/Akt/FOXO1a pathway. Although this pathway is well defined, many of the details remain unknown. Therefore, the exact target of the second category and the mechanism by which members inhibit the translocation of FOXO1a remains for further investigation.

It is too early to say whether one or more compounds from this screen can be developed into a cancer therapeutic. Excellent candidates for further development would be those compounds that are both potent inducers of apoptosis in malignant cells and nontoxic to normal cells. Even if most of the screened compounds do not end up in clinical use, they may still serve as good tools for laboratory use to help us understand the roles of their targets in tumorigenesis, and this may possibly lead to the discovery of new members of the pathway or better homologs for therapeutic purposes. Thus, the emergence of these PI3K/PTEN/Akt pathway inhibitors may have great impact on efforts to study signaling events and further development of anticancer agents.

It is worth remembering that the PI3K/PTEN/Akt pathway forms a complicated network with other signaling pathways that modulate cell survival and death, e.g., the MDM2/p53 pathway [2, 10, 17]. Akt phosphorylates MDM2 and activates its E3 ubiquitin ligase activity. Consequently, p53 is degraded more rapidly by MDM2 in cells with activated Akt. Inhibition of Akt function by the inhibitors may result in reduction of MDM2 activity and therefore an enhancement of p53 transcriptional activity. p53 can transcriptionally activate PTEN, which may further inhibit Akt activity. Therefore, inhibition of Akt by the inhibitors may trigger a positive feedback with perhaps additional anti-tumor effects. However, p53 is frequently mutated in human tumors, as is PTEN to a lesser extent. Fortunately, it may be much less frequent that both tumor suppressors are deficient in the same tumors. Screening for PTEN mimics or, broadly, PI3K/Akt pathway inhibitors may complement the efforts focusing on mutant p53 restoration [3, 18, 19]. It will be of interest in the not-too-distant future to determine the effects of the novel compounds in combination with other agents in human tumors and animal models with defined genetic alterations. It may also be of interest to investigate the effects of restoring nuclear expression of other Akt targets, such as p21 (WAF1/CIP1) [20], alone and in combination with the FOXO1a targeting agents. The progress made by Kau et al. [15] toward identifying a potential lead drug that targets a major pathway deregulated in cancer represents an important milestone for academic researchers and shows that it is possible to conduct unique and important drug discovery at an academic institution [21].

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Orthogonal Base Pairs Continue to Evolve

Recent developments in the design and construction of unusual analogs of the natural nucleic acid bases have reached a milestone with the report (in this issue of *Chemistry & Biology* [1]) of a new orthogonal base pair that allows site-specific introduction of a photocrosslinkable modified base into an RNA molecule by T7 RNA polymerase-mediated transcription of DNA containing the base-pairing partner.

One of the more interesting challenges in modified base design has been the creation of new orthogonal base pairs that would function as substrates for enzymes involved in the synthesis and processing of nucleic acids and would thereby provide a means for the expansion of the genetic code [2]. The earliest concerted effort to expand the genetic code and create new base pairs came from the laboratory of Benner and coworkers. In studies initiated during the 1980s, a series of putative base pairs containing alternative orthogonal hydrogen bonding patterns were designed and synthesized [3, 4]. Although the initial results with the isoC-isoG base pair looked promising, subsequent work showed that isoC was unstable in aqueous solution [5]. Furthermore, different polymerases show a broad range of specificity and recognition characteristics with these bases as template and as triphosphate substrates. In subsequent years the Benner laboratory continued to explore new variations on alternative H-bonding pattern modified bases. These studies included C-linked pyrimidine analogs [4, 6], which although optimal from the standpoint of alternative H-bonding patterns, proved to be unstable toward epimerization at C1′.

In a recent paper, Hutter and Benner now appear to have solved this problem [7]. 6-Amino-2-oxo-(1H) pyridine linked C3 to C1′ of 2′-deoxyribose is prone to epimerization through a mechanism initiated by protonation of the furanose oxygen followed by furanose ring opening coordinated with loss of the proton on N1. Reclosure leads to both the α and β epimers about C1′. Hutter and Benner rationalized that the introduction of a strong electron withdrawing group at C5 would prevent the electron release required for ring opening. Indeed they have now demonstrated that the iso-C analog, 6-amino-3-(2′-deoxy-β-D-ribofuranosyl)-5-nitro-(1H)-pyridine-2-one is stable to epimerization under conditions that one typically encounters during synthesis and manipulation of nucleoside triphosphates and oligonucleotides. One can expect that this work will soon be extended to polymerase-mediated nucleic acid replication.

As an alternative to orthogonal hydrogen bonding nucleoside analogs, Kool and coworkers have been exploring nonhydrogen-bonding isosteres of the natural bases [8]. A critical finding from this laboratory was that the hydrophobic shape-complementary purine-pyrimidine analogs preferentially pair with one another [9, 10]. Others variations on this theme have uncovered a number of interesting new base pairs. Most noteworthy has been the effort by Romesberg, Schultz, and coworkers, who have focused on the theme of creating unique hydrophobic base analogs with significant preference for self-pairing in nucleic acid duplexes [11].

Recently Hirao and coworkers reported a strategy for the construction of orthogonal base pairs that depend more on molding complementary shapes rather than hydrogen bonding pattern [12, 13]. They have found pairing specificity between a purine bearing a nonhydrogen-bonding substituent on C6 of a purine and a pyridine C-nucleoside that mimics a pyrimidine nucleoside minus the C4 amino or oxo group. This pairing is illustrated in