Restoring functions of tumor suppressors with small molecules

Developing small molecule inhibitors of oncoproteins, which are activated in tumor cells, is a newly popular strategy for cancer-related drug discovery. The complementary strategy, developing small molecules that restore the function of missing tumor suppressors, is much more difficult to realize. However, in this issue of *Cancer Cell*, Kau et al. report the discovery of small molecules that reverse some cellular consequences of the loss of the tumor suppressor *PTEN*.

In recent years, small molecules and antibodies targeted against oncogenic proteins have proven to be effective antitumor agents. These molecularly targeted agents, Gleevec, Herceptin, and Iressa, have raised hopes that an effective and generally applicable strategy for reversing tumor growth may be disruption of the biochemical functions of oncoproteins (Druker, 2002). These oncogenic proteins are produced by activating mutations in proto-oncogenes. Extensive resources are being deployed in efforts to discover small molecules or antibodies that inhibit oncoproteins, particularly those that function biochemically as kinases (Shawver et al., 2002).

This strategy would not appear, on its face, to be applicable to tumor suppressors, which are frequently mutated or deleted in tumor cells (Hansen and Cavenee, 1988). When a tumor suppressor protein is present in a mutated form, it is conceivable, albeit unlikely, that the normal function of the protein can be restored by a small molecule that binds to the mutant protein and causes an appropriate conformational change. However, when a tumor suppressor gene has been deleted from the genome of a tumor cell, using a small molecule to restore the function of the encoded tumor suppressor protein appears to be a near-impossible task. In such circumstances, because the encoded protein is entirely lacking from tumor cells, it is necessary to find a small molecule that replaces partially or completely the biochemical function of the missing protein.

In this issue of *Cancer Cell*, however, Kau et al. (2003) demonstrate that it is possible to restore at least one function of the tumor suppressor PTEN using small organic molecules in cells that lack PTEN. This result suggests a strategy for reversing the consequences of deletions of tumor suppressor genes, namely (1) determining the cellular functions of the encoded tumor suppressor proteins, (2) developing high-throughput screens for small molecules that restore these cellular functions via interaction with downstream proteins, and (3) elucidating the mechanisms of action of such compounds using information about signaling networks upstream and downstream of the missing tumor suppressor protein.

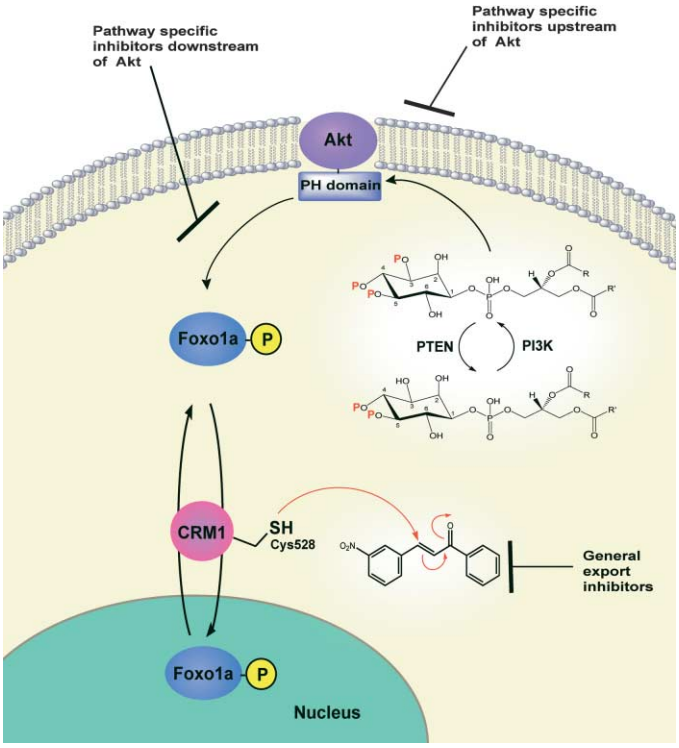

Figure 1. Chemical genetic analysis of PTEN-regulated Fox01a localization.

Kau et al. performed a high-throughput chemical screen for small molecules that localize Fox01a to the nuclei of PTEN-deficient cells. PTEN functions as a lipid phosphatase upstream of Akt, a kinase that phosphorylates Fox01a and thereby regulates its nuclear localization. The screen revealed both compounds that were PTEN/Akt/Fox01a-pathway-specific and compounds that were general nuclear export inhibitors. The pathway-specific compounds therefore reverse at least one consequence of PTEN deficiency in tumor cells.

The tumor suppressor protein selected for study by Kau et al. was PTEN, a lipid phosphatase that is a negative regulator of PI3K- and Akt-driven cell proliferation (Sulis and Parsons, 2003). PTEN functions biochemically by dephosphorylating lipid phosphates that are products of PI3K phosphorylation. FOXO1a restrains cell growth and it is normally unable to do so in the absence of PTEN (Burgering and Medema, 2003).

In PTEN null cells, FOXO1a is inactivated by PI3K-dependent phosphorylation; Akt phosphorylates FOXO transcription factors at multiple sites. Phosphorylated FOXO1a is inappropriately localized to cytoplasm and prevented from restraining cell cycle progression. Kau et al. reasoned that relocalization of FOXO1a to the nucleus would reverse consequences of PTEN deletion and the tumorigenicity of PTEN null cells. These investigators performed a high-throughput chemical screen to identify inhibitors of nuclear export of FOXO1a in PTEN-deficient tumor cells. In these cells, transfected FOXO1a has a cytosolic localization. Compounds that relocalized FOXO1a to the nucleus were discovered and are of interest for their ability to possibly reverse consequences of PTEN deficiency.

The authors found a series of compounds, from among 18,000 tested, exhibiting the desired activity. These compounds were categorized in sec-

ondary screens as (1) general nuclear export inhibitors or (2) PI3K/Akt pathway-specific export inhibitors. Many of the general export inhibitors reacted with the nuclear export receptor CRM1, which binds nuclear export sequences of proteins and exports them into the cytoplasm. A known inhibitor of CRM1 is leptomycin B, which inactivates CRM1 by covalently labeling cysteine 528 (Kudo et al., 1999; Nishi et al., 1994).

The general export inhibitors interfered with CRM1 function and nonspecifically blocked export of proteins with nuclear export sequences. All 19 general export inhibitors were found to target cysteine 528 in CRM1, in a manner seemingly identical to leptomycin B. Inspection of the chemical structures of these compounds reveals that they contain electrophilic functionalities, such as Michael acceptors, just as leptomycin B does. This important observation suggests that in the future, compounds found to inhibit export of proteins should be inspected for α,β-unsaturated carbonyls, or other thiol-reactive electrophilic functionality, to determine whether such compounds may be acting as CRM1 inhibitors.

The pathway-specific FOXO1a-export inhibitors inhibited FOXO1a export but did not alter export of an unrelated protein. Additional epistasis experiments revealed that some of these compounds act upstream of Akt and others act downstream of Akt. This chemical genetic screen and subsequent analysis is an example of the utility of the chemical genetic approach (Stockwell, 2000) in which the functions of novel small molecules are analyzed systematically by measuring their affects on proteins and signaling pathways. This approach is strengthened by exploiting knowledge about existing biologically active molecules and comparing them to newly identified compounds (Root et al., 2003). Moreover, the use of a phenotypic change as an output enables identification of small molecules that target multiple components of a signaling pathway and yet result in a desired effect.

Thus, the comparison of compounds identified in the primary screen to the existing nuclear export inhibitor leptomycin B revealed a CRM1-dependent mechanism of action for numerous general export inhibitors. Because many of these compounds are synthetically prepared small molecules with few stereogenic centers, they may serve as more accessible, less toxic, and more stable leptomycin B substitutes. In addition, they provide scaffolds and cysteine-reactive functional groups that can be used to develop potent CRM1 inhibitors.

Systematic classification of identified FOXO1a nuclear export inhibitors based on their behavior in secondary assays allowed Kau et al. to determine the role of calmodulin in regulating FOXO1a signaling. The authors discovered that calmodulin inhibitors such as phenothiazines identified in the screen, along with structurally unrelated calmodulin inhibitors W-13, calmidazolium, and ophiobolin A, relocalized FOXO1a to nucleus. These results highlight the value of screening both known and novel compounds; such a strategy facilitates identification of molecular targets and provides a basis for structure-function analysis.

An important aspect of these investigations is that they involved both chemists and biologists in the analysis of the screen. Such cooperation proved essential to the correct interpretation of the mode of action of the general export inhibitors; the recognition of Michael-type electrophilic functionality likely suggested these compounds should be tested for their dependence on cysteine 528 in CRM1. Although the notion of multidisciplinarity is often touted, it is rarely implemented, due to institutional and cultural barriers. Those groups that are able to surmount such chasmal divides will be the ones to reap the benefits of the chemical genetic approach.

Finally, these investigations demonstrate that small molecules can be used to reverse consequences of the loss of a tumor suppressor gene. By selecting compounds that restore nuclear FOXO1a localization in PTEN null cells, Kau et al. were able to identify small molecules that target multiple steps in the PI3K/PTEN/Akt signaling pathway. Such an unbiased screening approach is particularly valuable for exploring novel targets for therapy within signaling pathways. Because loss of tumor suppressors is a major component of tumorigenicity, this strategy might prove to be a powerful one for reversing the consequences of genetic changes in tumor cells.

Inese Smukste and
Brent R. Stockwell*

Whitehead Institute for Biomedical Research
Nine Cambridge Center
Cambridge, Massachusetts 02142
*E-mail: stockwell@wi.mit.edu

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