

during differentiation of myeloid cells. Gadhoum and Sackstein also showed that the majority of CD15 expressed in differentiated neutrophils is present on two glycoproteins: CD43 and PSGL-1. This is a rather surprising finding given that CD15 is abundantly present in glycolipids⁸. Considering that CD43 and PSGL-1 are extended from the plasma membrane, it is important to determine how Neu-1 reaches the end of such extended molecules.

The conversion of CD15s to CD15 may also have significance in differentiation and maturation of other hematopoietic cells. Neutrophil progenitor cells are thought to adhere to bone marrow stroma probably using a CD15s–PSGL-1 interaction⁹. Similarly, immature T lymphocytes bind to thymus stroma cells

through interaction of CD15s and PSGL-1 (ref. 10). Differentiation and maturation of these progenitor cells and immature cells may also be associated with switching of adhesive counterparts through removing sialic acid by endogenous sialidase. In another exemplary system, the B-cell-specific lectin CD22 binds to $\alpha(2-6)$ -linked sialic acid, and B-cells deficient in the enzyme that forms $\alpha(2-6)$ -linked sialic acid show suppressed B-cell receptor complex signaling^{11,12}. It is possible that endogenous sialidase removes this terminal sialic acid and thereby regulates B-cell receptor complex-mediated signaling. The work by Gadhoum and Sackstein shows that these and perhaps other novel functions of endogenous sialidase are yet to be explored.

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Bcl-2 turns deadly

Bing Qi & J Marie Hardwick

Small-molecule inhibitors of anti-apoptotic Bcl-2 proteins and BH3 mimetic peptides are promising anticancer agents. A recent study identifies a Nur77-based peptide that converts anti-apoptotic Bcl-2 proteins into pro-apoptotic molecules, providing another potential cancer therapeutic strategy.

Bcl-2 family proteins are frequently deregulated in tumors and can confer resistance to therapeutic agents. Although their mechanisms of action are still under investigation, Bcl-2 family proteins are key regulators of mitochondrial cell death pathways. In damaged cells, three subsets of Bcl-2 family proteins interact to determine cell fate. The anti-death Bcl-2 family members (Bcl-2, Bcl-x_L) compete with specific members of the pro-death BH3-only subset (Bid, Bim, Bad) to prevent oligomerization of the membrane-permeabilizing family members that damage mitochondria and kill cells (Bax, Bak). A wide variety of therapeutic strategies have been developed to target Bcl-2 family proteins, including BH3 mimetic peptides derived from the BH3 domains of Bim and of caspase-cleaved Bid (tBid) that bind and activate Bax to kill cells¹. The small-molecule inhibitor ABT-737 was designed to mimic the BH3 domain of Bad by binding in a cleft on the front of Bcl-2 and Bcl-x_L and inactivating their anti-apoptotic function². However,

because N-terminal truncation of Bcl-2 and Bcl-x_L by caspase cleavage can convert these anti-apoptotic factors into potent pro-death molecules^{3,4}, it is theoretically possible to exploit this event as a therapeutic strategy. In a recent report, Kolluri *et al.*⁵ found that a short peptide corresponding to the nuclear orphan receptor Nur77 binds to and converts Bcl-2 into a pro-death molecule. This finding opens the door for the development of small molecules with similar actions for the treatment of cancer.

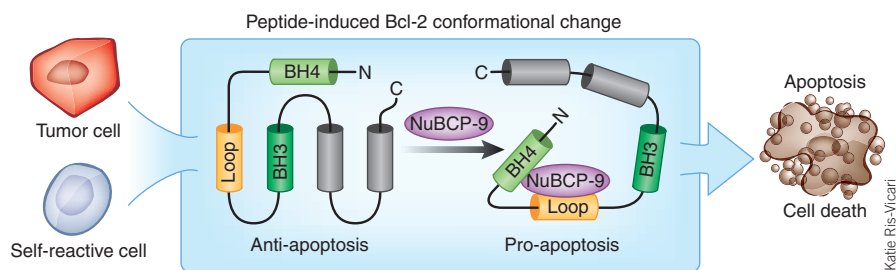
Nur77 and related orphan nuclear receptors are implicated in the elimination of autoreactive T cells, brain development and other processes⁶. Following up on their earlier report that Nur77 induces apoptosis by binding and converting Bcl-2 into a pro-apoptotic factor, Kolluri *et al.*⁵ found that a nine-amino-acid peptide (NuBCP-9) that corresponds to a region of Nur77 required for interaction with Bcl-2 is also pro-apoptotic. NuBCP-9 and its enantiomer bind the N-terminal BH4 domain and adjacent unstructured loop domain of Bcl-2, an important regulatory region where phosphorylation and caspase cleavage occurs. The authors conclude that NuBCP-9 works through a Bcl-2-dependent mechanism to induce cell death by showing that Bcl-2 knock-out fibroblasts (*bcl-2*^{-/-} MEFs) are more resistant to NuBCP-9-induced apoptosis. Fitting

with this model, but contrary to the norm, Bcl-2 overexpression enhances NuBCP-9-induced cell death in T-cell leukemia-derived cells. Importantly, injection of NuBCP-9 peptide suppresses growth and induces apoptosis in tumor cells xenografted in mice.

How does the NuBCP-9 peptide convert Bcl-2 into a pro-death factor? Kolluri *et al.*⁵ show that direct binding of NuBCP-9 to Bcl-2 induces conformational changes in the Bcl-2 protein (Fig. 1), detected by shifts in biophysical properties and by exposure of an antibody epitope in the BH3 domain of Bcl-2. Exposure of the BH3 helix in tBid and Bax is critical for their pro-apoptotic function, and this mechanism appears to be conserved during the conversion of Bcl-2 to its pro-apoptotic conformation. The authors provide evidence that NuBCP-9-converted Bcl-2 does not adopt the membrane-permeabilizing function of Bax, and does not mimic the BH3-only proteins Bid or Bim, which can directly activate Bax. Instead, converted Bcl-2 mimics a different group of BH3-only proteins (for example, Bad) that bind to and inactivate the anti-death protein Bcl-x_L, thereby releasing the brakes on tBid activation of Bax to kill cells¹. These experimental systems are complex, however, so other possible mechanisms should be explored.

The results of Kolluri *et al.*⁵ suggest a mechanism in which a major conformational change

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Figure 1 Small molecules convert anti-death factors into pro-death factors. A peptide derived from Nur77 (designated NuBCP-9) converts anti-death Bcl-2 and Bcl-B into pro-death factors that activate Bax- and Bak-dependent cell death¹⁰. NuBCP-9 binds the anti-apoptotic conformation of Bcl-2 in the N-terminal loop domain and induces a conformational change. In the latter conformation, the BH3 domain is exposed and Bcl-2 promotes apoptosis.

that likely involves both the N and C termini of Bcl-2 serves as a molecular switch between anti-death and pro-death activities. The ability of NuBCP-9 to flip this switch is likely to be distinct from the mechanism of the BH3 mimetic ABT-737 now in clinical trials. While ABT-737 kills tumor cells by binding and inactivating Bcl-2 and Bcl-x_L, it also unexpectedly protects neurons from hypoxia-induced synaptic decline and from increased mitochondrial permeability, which implies that ABT-737 also inactivates the pro-death function of Bcl-x_L and perhaps also Bcl-2 (ref. 7). In contrast, the Nur77 mimetic peptide NuBCP-9 inhibits only

the anti-death conformation to promote the switch to a pro-death form of Bcl-2, potentially enhancing its antitumor effects.

Does Bcl-2 conversion to a pro-death factor also occur during physiological processes, and does Nur77 mediate the conversion of Bcl-2 to a pro-death factor in animals? Interestingly, Thompson and Winoto⁶ recently reported that Nur77 translocation to mitochondria and conversion of Bcl-2 into a BH3-exposed killer protein occurs during elimination of autoreactive T lymphocytes using two different mouse models. These observations imply a physiological role for Nur77-induced pro-death activ-

ity of Bcl-2, though a causal role for Bcl-2 in negative selection has not yet been established. Because the Nur77 mimetic NuBCP-9 does not target Bcl-x_L, it will avoid the complicating side effects of thrombocytopenia associated with ABT-737, but future studies will be required to determine whether NuBCP-9 causes immunosuppression.

Another important question is whether this death conversion of Bcl-2 can be harnessed to treat cancer. The particularly potent effects of NuBCP-9 against Bcl-B provide a reasonable target, as Bcl-B was reported recently to be elevated in several human cancers, where it is thought to confer resistance to chemotherapeutic drugs and irradiation therapy⁸. Efforts are now underway to identify small molecules that mimic the effects of the Nur77 peptide NuBCP-9 (ref. 9).

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Short-circuiting RNA splicing

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Isoginkgetin has been identified as a general inhibitor of pre-mRNA splicing using an *in vivo* screening assay. This and related inhibitors will not only be useful as tools to decipher the roles of the individual components of the spliceosome but may also serve as therapeutics.

The spliceosome is a complex ribonucleoprotein machine that is responsible for the maturation of cellular pre-mRNAs by removing introns and ligating together the surrounding coding regions, called exons. This process is known as RNA splicing and is tightly regulated to determine what protein isoforms are created from a pre-mRNA. Incorrect splicing of RNA can cause diseases

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such as β -thalassemia and inherited breast cancer. Despite the importance of RNA splicing, much about the inner workings of the spliceosome is unknown. Studies aimed at understanding spliceosomal function could be aided with small molecules that modulate the activity of the spliceosome, using chemical genetics approaches and cell-based systems to systematically study the splicing machinery. A recent paper in the *Journal of Biological Chemistry* by O'Brien *et al.*¹ describes a cell-based screen to identify small molecules that affect pre-mRNA splicing and reports that isoginkgetin is a general inhibitor. Armed with such compounds, we can begin to unravel the complexities of the spliceosome.

Using small molecules or chemical genetics probes to study the spliceosome is an attractive approach partly because the spliceosome is a multisubunit macromolecular machine and thus has folding behaviors that depend on the assembly of the entire system. Therefore, employing RNA interference or conventional genetics to knockout a particular protein to determine its function may not be useful. Fortunately, academic investigators have access to high-throughput screening facilities to facilitate the identification of chemical probes to study biological function. Small molecules that perturb biological function are well suited for studying macromolecular machines because they allow one to decipher the roles of individual components