Stapled peptides can open routes to new therapy approaches for multiple diseases

Stapled proteins formed by a technique called hydrocarbon stapling have been shown to activate apoptosis and induce cancer cell death in vivo. Programmed cell death or apoptosis is needed to destroy cells that are a threat to the integrity of the organism. Apoptosis comes into play in the following circumstances:

- Cytotoxic T-lymphocytes (CTL) kill virus-infected cells by inducing apoptosis
- As cell-mediated immune responses wane, the effector cells need to be removed to prevent them from attacking body constituents. Defects in apoptosis machinery in the cell can cause autoimmune diseases such as lupus erythematosus and rheumatoid arthritis.
- Cells with damaged DNA can lead to cancer. Damaged cells produce more p53 that induce apoptosis.
- Radiation and chemotherapy induce apoptosis in certain cancer cells.

Inactivation of apoptosis is believed to be the main reason leading to the development of cancer. Response to cancer therapy is also governed by apoptotic responses. In some cases it has been observed that apoptosis is not the main mechanism for the death of cancer cells in response to common treatment regime, thus leading to resistance to such treatment regime.

Apoptosis is a highly regulated, energy-dependent program, whereby the cell activates a signaling cascade that leads to cell death without triggering an inflammatory response. Apoptosis is mediated by two distinct pathways – extrinsic and intrinsic cell death pathways. The extrinsic pathway is activated when death ligands, such as Fas ligand or TNF-α, bind to their cognate receptors at the plasma membrane. This causes homotrimerization of the receptor and recruitment of specific adaptor proteins, such as Fas-associated death domain and procaspase-8, into a death-inducing signaling complex. This, in turn, leads to activation of initiator caspase-8, which subsequently activates effector caspases. In case of the intrinsic pathway, the mitochondria play a central role in the integration and execution of a wide variety of apoptotic signals, including loss of growth factors, hypoxia, oxidative stress, and DNA damage. The mitochondria provide the energy required for execution of the apoptotic program and release of proapoptotic

proteins such as cytochrome *c*, endonuclease G, and apoptosis-inducing factor. Release of cytochrome *c* leads to apoptotic protease-activating factor (Apaf-1)-mediated activation of initiator caspase-9, which in turn activates effector caspases. Thus the extrinsic and intrinsic pathways have different initiator caspases but converge at the level of the effector caspases.

The intrinsic pathway of apoptosis is regulated by members of the Bcl-2 family as shown in figure 1. (Fig.1) This family is composed of pro- and antiapoptotic proteins that share up to four conserved regions known as Bcl-2 homology (BH) domains. Antiapoptotic members such as Bcl-2 and Bcl-X_L contain all four subtypes of BH domains and promote cell survival by inhibiting the function of the proapoptotic Bcl-2 proteins. Antiapoptotic Bcl-2 proteins have been reported to protect cells from many different apoptotic stimuli and are important for cell survival. In some circumstances, Bcl-2 and Bcl-X_L are targets of caspases, and cleavage of these proteins converts them from prosurvival to proapoptotic molecules that are able to induce cytochrome *c* release from the mitochondria.

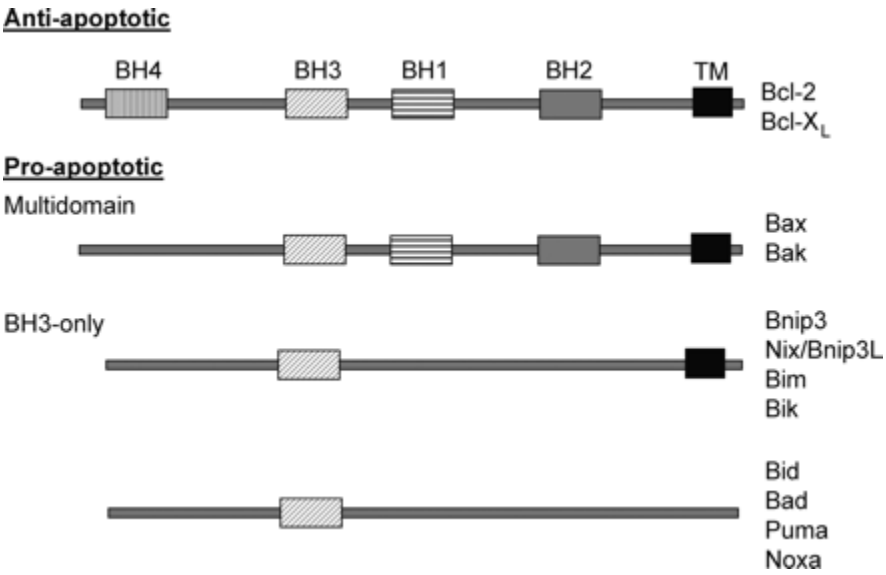

Fig. 1. Domain structure of Bcl-2 family proteins. Bcl-2 homology (BH) and transmembrane (TM) domains are indicated.

BH3 is a spring-like shaped α helix with amino acids on its surface that bind to and inhibit anti-death proteins such as BCL-2, as well as activating pro-death proteins under certain

circumstances. However, when BH3 is produced synthetically without its parent protein, its shape is lost and its functionality impaired.

To stabilize the peptide, Loren D Walensky and colleagues at the Dana-Farber Cancer Institute used a chemical strategy called the hydrocarbon-stapling technique developed by chemist colleague Gregory Verdine. Some amino acids in the natural sequence are replaced with synthetic ones bearing hydrocarbon 'tethers'. These link to form chemical 'staples', which reinforce the α-helical structure. The new peptide retains its biological activity and actually binds more strongly to the BCL-2 target, the researchers report. The stapled peptides, called "stabilized alpha-helix of BCL-2 domains" (SAHBs), were helical, protease-resistant, and cell-permeable molecules that bound with increased affinity to multidomain BCL-2 member pockets. A SAHB of the BH3 domain from the BID protein specifically activated the apoptotic pathway to kill leukemia cells. In addition, SAHB effectively inhibited the growth of human leukemia xenografts in vivo. Hydrocarbon stapling generated BH3 peptides with improved pharmacologic properties. Different BCL-2 proteins are implicated in different cancers, but all contain α-helical BH3 domains. 'There may be a therapeutic window for BH3 molecules in cancers that specifically exploit cell death pathways for survival,' says Walensky. 'If you can inhibit an anti-death protein distinctly required for the cancer but not singly necessary for a normal cell's survival, you may be able to trigger cell death in the cancer while leaving normal cells unharmed.'

'This is a proof of concept that you can turn on the death-promoting proteins with a peptidomimetic,' says Steve Dowdy of the Howard Hughes Medical Institute at the UCSD School of Medicine, La Jolla, USA whose perspective article accompanied the Walensky paper. 'More broadly it shows that mimicking protein–protein interactions can be therapeutically beneficial in preclinical models, and we should be able to apply this in other pathways too.' He adds that more work to investigate potential toxicity and solve the problem of delivery will be needed before the approach can be tried in humans.

The α-helix motif occurs frequently in biologically important protein interactions, so stapling might allow peptides to be used as drugs in many different applications. 'If we could target protein interactions at critical biological control points using the natural sequence for a protein

target, we might have a whole new set of tools to study and manipulate protein interactions within cells', Walensky says.

Thus hydrocarbon stapling of native peptides may provide a useful strategy for experimental and therapeutic modulation of protein-protein interactions in many signaling pathways.

References:

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