Chemotherapy: Open Access
ISSN: 2167-7700

Johanning, Chemotherapy 2012, 1:3
DOI: 10.4172/2167-7700.1000e109

Editorial Open Access

Stapled Peptides as Anti-Apoptotic Drugs

Gary L. Johanning*

Associate Professor, University of Texas, USA

A major problem with many cancer chemotherapeutic drugs is their lack of selectivity. They attack and kill not only the cancer target of interest, but in many cases also normal cells in tissues that are vital for cancer patient survival. Drugs that target cellular functions that are essential specifically for cancer cell survival should thus be of benefit to the cancer patient undergoing chemotherapy.Cancer cells have adapted numerous strategies to avoid apoptosis, or programmed cell death. The BCL-2 family contains anti-apoptotic and pro-apoptotic proteins. The anti-apoptotic BCL-2 family proteins have been linked to the survival of pathogenic cells that include cancer cells. RNA inhibition has been one approach to blocking anti-apoptotic BCL-2 and hence cancer cell survival. However, there is a question about the effectiveness of siRNA in long-term gene silencing.

An alternative approach that has emerged to modulate, either positively or negatively, the activity of apoptotic signaling proteins is the use of stapled peptides. Peptides derived from native protein sequences are unfolded, and tend to shift conformations between helices, sheets and random coils, because they have been removed from the stabilizing influences of the protein milieu in which they naturally reside. The regularity of the α helical backbone conformation in the peptide can be maintained by introducing non-natural amino acids into the peptide sequence; the non-natural amino acids are incorporated on adjacent turns of the α-helix. A covalent bond ("staple") between non-natural amino acid residues containing terminal double bonds is formed by a ruthenium-catalyzed metathesis reaction that closes the ring, tethers the two non-natural amino acids and stabilizes the peptide in the α-helical configuration. The use of alkyl tethers to produce an all-hydrocarbon chain linking the non-natural amino acids greatly stabilized the helical peptide, in comparison to non-hydrocarbon substituents. The use of an all-hydrocarbon cross-linker was a key development that greatly improved cell uptake of the peptides, as well as their resistance to protease degradation and their effectiveness in modifying cancer cell survival. These are all qualities that are highly desirable in a chemotherapeutic agent.

Loren Walensky's laboratory has produced stapled peptides targeting a variety of proteins in the BCL-2 network. While working with Stanley Korsmeyer, Walensky and co-workers reported that BCL-2 stapled peptides induce apoptosis after binding to BCL-2 domains *in vivo* [1]. In a more recent study by Walensky'sgroup, a library of BCL-2 domains modeled after the BH3 domains of human BCL-2 family proteins was produced, with a goal of identifying BH3 domains that would selectively inhibit the protein MCL-1 [2]. MCL-1 is one of a group of anti-apoptotic proteins, including BCL-2 itself, that become overexpressed in cancer, and promote survival of cancer cells by sequestering the alpha-helical BCL-2 homology domain 3 (BH3) of pro-apoptotic BCL-2 family members; the BH3 domain is essential for the killing function of pro-apoptotic proteins. A selective MCL-1 inhibitor, as opposed to a selective inhibitor of alternative anti-apoptotic proteins, was studied for several reasons. MCL-1 is overexpressed in several hard-to-treat cancers, it blocks the activation of pro-apoptotic proteins and it plays an important role in resistance to apoptosis. In addition, a small-molecule BH3 mimetic that specifically targeted BCL-2, which did induce apoptosis in some cancers, was found to be ineffective against cancer cells that overexpress MCL-1.

The peptides in the MCL-1 library were stapled in a manner such that they were tethered to the non-interacting face of the peptides, and the stapled peptides were termed "stabilized alpha-helix of BCL-2 domains" (SAHBs).To enrich for domains that selectively inhibit MCL-1, the libraries were screened for binding to recombinant proteins that specifically eliminated the BH3-only proteins NOXA, PUMA, BID and BIM, as well as the pro-apoptotic protein BAK. After eliminating these proteins, fluorescence polarization assays were used to measure how strongly the fluorescently labeled SAHBs bound to a deletion construct of recombinant human MCL-1 that contained the BH3-binding pocket.

How effective were the SAHBs in inhibiting MCL-1? The authors found that the BH3 helix from MCL-1 itself showed exquisite selectivity for the helix-binding domain of the MCL-1 protein. One such helix, MCL-1 $SAHB_A$, selectively and strongly (K_D, 43 nM) bound to the BH3-binding pocket of MCL-1. When a single selectivity-determining point mutation was made in this helix, it was no longer selective for MCL-1, but instead bound to both MCL-1 with a lower affinity (K_D, 191 nM) and to BCL-X_L (K_D, 89 nM). The crystal structure of the complex between another MCL-1 SAHB, namely MCL-1 $SAHB_D$ (which showed the strongest interaction of all the MCL-1 SAHBs: K_D, 10 nM), and the MCL-1 BH3-binding pocket revealed that this SAHB is indeed an alpha-helix that binds via hydrophobic interactions to the BH3-binding groove at various points along the helix. A particularly interesting finding was that the hydrophobic staples themselves contribute to the interaction, and the authors indicated that optimal staple design and hydrophobicity might improve the pro-apoptotic effectiveness of the SAHBs without interfering with their specificity.

In this regard, pro-apoptotic effects were demonstrated for the MCL-1 $SAHB_D$ after its addition to mitochondria exposed to the BID BH3; this action heightened BAK-mediated cytochrome C release. *In vitro* relevance was additionally demonstrated in multiple myeloma cells, where MCL-1 $SAHB_D$ was able to dissociate MCL-1–BAK complexes in these cells. MCL-1 $SAHB_D$also dose-responsively sensitized Jurkat cells to both of the extrinsic pathway activators TRAIL and Fas ligand, suggesting that this SAHB sensitizes cancer cells to MCL-1–dependent inducers of apoptosis.

The efficacy of peptide therapeutics could be greatly improved using peptide stabilization approaches such as peptide stapling. Stapled peptides have several appealing features as agents for cancer chemotherapy. First, they can very specifically bind to and interfere with the function of anti-apoptotic proteins in the cell. A variety of drugs are used to alter apoptosis in cancer, but in many instances they

***Corresponding author:** Gary L. Johanning, Associate Professor, University of Texas, USA, Tel: 512-332-5211; Fax: 512-332-7375; E-mail: gljohann@mdanderson.org

Received April 28, 2012; **Accepted** April 30, 2012; **Published** May 02, 2012

Citation: Johanning GL (2012) Stapled Peptides as Anti-Apoptotic Drugs. Chemotherapy 1:e109. doi:10.4172/2167-7700.1000e109

are not highly specific for a single target, as the stapled peptides appear to be. Second, peptides are relatively inexpensive, with the caveat that the addition of hydrophobic staples would boost the cost somewhat. Third, peptides are more cell permeable than antibodies and some chemotherapeutic drugs. This raises the question of whether an arsenal of stapled peptides should be generated against a larger number of cancer targets, in addition to pro- and anti-apoptotic proteins.

There are, of course, some unanswered questions and areas that still need to be addressed for MCL-1 SAHBs. Are they effective *in vivo*? This will likely be pursued in xenograft mouse models and eventually in human clinical trials. Will cancer cells remain sensitive to SAHBs? The major shortcoming of cancer chemotherapy is development of resistance, and there is little or no information about whether patients will become resistant to SAHBs.There are also critical questions related to the stability and longevity of the peptides in the serum.

In summary, chemotherapy regimens based on stapled peptides that bind to cancer targets have great potential to benefit the cancer patient, because these reagents exhibit superior specificity in interacting with the target, and can be selected to either augment or inhibit target activity in a manner favorable to the patient.

References

1. Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, et al. (2004) Activation of apoptosis *in vivo* by a hydrocarbon-stapled BH3 helix. Science 305: 1466-1470.

2. Stewart ML, Fire E, Keating AE, Walensky LD (2010) The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer. Nat Chem Biol 6: 595-601.