Bioactive Cell-Penetrating Peptides: Kill Two Birds with One Stone

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Jones et al. (2010) propose an innovative strategy for the development of bioactive cell-penetrating peptides. They combine computer-based design with specific targeting to elaborate a potent cell-penetrating bioactive peptide derived from cytochrome C. This short chimera peptide induces tumor cell apoptosis by targeting and sequestering nucleoporin, a key component of the nuclear pore complex.

Cell proliferation and programmed cell death are controlled by a network of both posttranslational protein modifications and protein/protein interactions, which are essential to maintain the spatiotemporal activation of the different pathways within the cell. Although small molecules remain the major drugs used in clinic, in numerous cases their therapeutic impact has reached limitations due to their insufficient capability to reach targets, lack of specificity, requirement for high doses leading to toxicity, and major side effects (Utreja et al., 2010). Therefore, targeting protein/protein interfaces involved in the development of pathologies has been proposed as a potent strategy to overcome these limitations and the lack of specificity of the currently used therapeutic molecules. Several small peptides have been described to block protein/protein interactions in vitro and in cellulo and to modulate cell cycle aberrant proliferation, apoptosis, and viral infection (Agopian et al., 2009; Rizzolio et al., 2010). The use of large therapeutic molecules such as peptides offers several advantages, including specificity, high potency, and large contact interfaces with their target, thereby limiting the emergence of drug resistance and improving important flexibility for sequence optimization and stabilization. But in counterpart, the pharmaceutical potency of these molecules remains restricted by their low bioavailability in vivo and by their poor cellular uptake, making the issue of delivery a keystone for their therapeutic development.

Several nonviral technologies have been designed to improve cellular uptake of therapeutic molecules. Twenty years ago, the concept of protein transduction domain (PTD) or cell-penetrating peptide (CPP) was proposed based on the observation that some proteins, mainly transcription factors, shuttle within cells and from one cell to another (Heitz et al., 2009). CPPs constitute very promising tools for noninvasive cellular import of cargos and have been successfully applied for ex vivo and in vivo delivery of a large set of therapeutic molecules, varying from small chemical molecules, nucleic acids, proteins, peptides, liposomes, and nanoparticles. CPPs are either chemically linked to the peptide drug or form stable, noncovalent complexes with cargos for cellular internalization (Heitz et al., 2009). CPPs have successfully delivered peptides, antibodies, and proteins to target different diseases, including cancer, asthma, apoptosis, ischemia, and stimulating cytotoxic immunity and diabetes in cellulo and in vivo at the laboratory level as well as in clinical trials (Wadia and Dowdy, 2005; Heitz et al., 2009).

Although the low potency of peptide drugs to enter cells has been partially solved by the use of CPPs, the complexity remains once additional sequences are required to control cellular trafficking and targeting. Moreover, limitations can also arise from the risk of introducing an exogenous carrier peptide sequence in the cells. Therefore, defining peptides that can enter the cell, specifically access a cellular target, and induce a strong biological response specific for targeted cells will be a major impact for therapeutic application of peptide drugs. The combination of structural and biophysical investigations together with cell biology studies has revealed that the size, the net charge, and structural versatility of CPPs constitute critical parameters to take into account for their potency and cellular uptake. Recent development in computer-based design and molecular modeling of peptides have identified several common features and provided new perspectives for in silico and de novo design of CPPs (Thomas et al., 2006; Hallbrink et al., 2005). Hallbrink et al. (2005) have proposed a Quantitative Structure Activity Relationship (QSAR)-algorithm based on a series of well-known CPPs as a means of predicting directly from the primary sequence of either natural proteins or artificial peptides, polycationic cell-penetrating peptide motifs. In this issue of *Chemistry & Biology*, QSAR-algorithm was successfully applied by Jones et al. (2010) in order to elaborate bioactive cell permeable peptides that mimic the role of cytochrome C a key regulator protein in programmed cell death (Yamaguchi and Perkins, 2009). Using human Cytochrome C as a protein template, 31 potential CPPs were identified, all derived from helical motifs and mainly the helix at the C terminus of the cytochrome C, which constitutes the most thermodynamically favorable structure for cell-membrane crossing. A potent peptide covering residues 77-101 $Cyt^{77\text{-}101}$ has been reported to efficiently enter the cell and to mimic the apoptogenic activity of native human cytochrome C by inducing moderate apoptosis.

Quite often, the low ratio between cellular uptake and the corresponding biological response is directly linked to the low potency of the peptide to escape

from the endosome and/or its poor ability to reach the target within the cell. It is therefore not only important to increase selectivity and specificity of therapeutic molecules, but also essential to improve their cellular trafficking to the target. Jones et al. (2010) have proposed a chimera peptide combining the highly penetrating active peptide $Cyt^{77\text{-}101}$ together with a nona-peptide containing the motif "FXFG" derived from the C terminus of nucleoporin 153 (Nup153). The goal was to target the FG-nucleoporin Nup153, one of the key players in the control of nuclear import and the selectivity of the nuclear pore complex NPC (Stewart, 2007). The Nup153-Cyt-C peptide exhibits a strong pro-apoptotic activity at submicromolar concentration, which is about 100 fold higher than the parental peptide. From a mechanistic point of view, although different targets seem to be involved, Nup153-Cyt C sequesters Nup153 at the periphery of the nuclear envelope and significantly disorganizes nuclear pore component architecture, thereby triggering apoptosis. The presence of Nup153 targeting sequence not only increases the peptide efficiency, but also its cellular uptake and endosomal release, which is due to an increase in the cationic and aromatic character of the peptide.

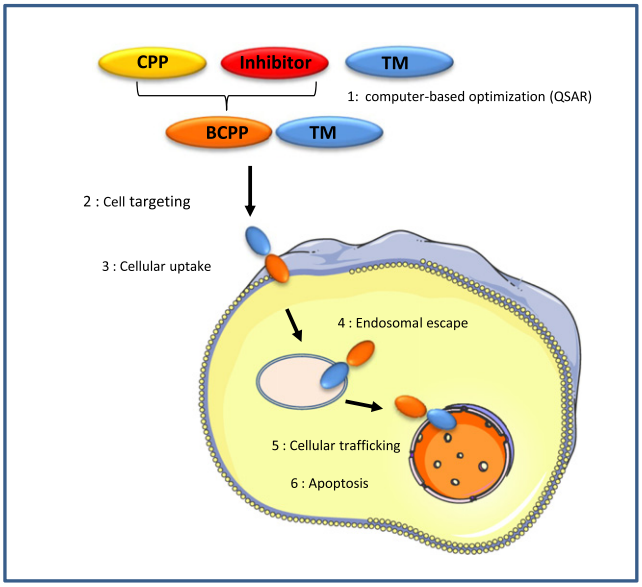

Figure 1. Design and Potency of Bioactive Cell-Penetrating Peptides

Cell-penetrating peptide (CPP) can be linked to inhibitor and targeting motif (TM) or computer-based optimized using (QSAR) (1) to a single sequence containing bioactive cell-penetrating sequence (BCPP) that will improve cellular uptake (3), endosomal release (4), and biological response (6), and a targeting motif (TM) that will favor either cell targeting (2) or cellular trafficking (5).

Jones et al. (2010) describe a very promising strategy for identification of potent protein/protein interface cell-penetrating peptide inhibitors (Figure 1). Applying this strategy to computer-based design of mimics of cytochome C, they have identified a potent peptide drug and suggest that nuclear pore organization constitutes an interesting new target for induction of apoptosis. Having only one peptide sequence that can inhibit a specific cellular pathway, enter cells, and be further modified for in vivo targeting will open new perspectives for the clinical application of peptides.

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