Review

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Cell-penetrating peptides: strategies for anticancer treatment

Drazen Raucher and Jung Su Ryu

Department of Biochemistry, University of Mississippi Medical Center, Jackson, MS 39216, USA

Cell-penetrating peptides (CPP) provide an efficient strategy for the intracellular delivery of bioactive molecules in various biomedical applications. This review focuses on recent advances in the use of CPPs to deliver anticancer therapeutics and imaging reagents to cancer cells, along with CPP contributions to novel tumor-targeting techniques. CPPs are now used extensively to deliver a variety of therapeutics, despite lacking cell specificity and having a short duration of action. Resolution of these shortcomings to enable increased cancer cell and/or tumor specificity could improve CPP-based drug delivery strategies, expand combined drug delivery possibilities, and strengthen future clinical applications of these peptides.

The challenge of intracellular drug delivery

The targeted delivery of drugs to specific intracellular locations so as to engineer cancer cell death represents a major research effort. However, an important obstacle to such targeted delivery has been the inability of chemotherapeutics to pass through protective, physiological barriers in tumor tissues. The cellular plasma membrane, despite its selective permeability to molecules essential to cell function and survival, has constituted a particularly daunting barrier. Therefore, the successful, active transport of drugs or drug carriers to intracellular targets (via vector molecules, such as peptides) requires the development of approaches that can accomplish cellular membrane translocation. Recently, a variety of approaches, including low-molecular-weight prodrugs, liposomes, and micro- and nanoparticles, has been developed and used to deliver therapeutics with varying degrees of success. Designed to improve pharmacokinetics and stay longer in the blood circulation, these drug delivery carriers represent a substantial advance in the design of improved approaches to tissue and cellular uptake [1,2]. Among the new approaches, the exploitation of the CPP (see Glossary), also known as protein transduction domains (PTD), currently affords one of the most frequently used means for achieving the efficient, intracellular delivery of a variety of cargos [3].

A CPP is a short peptide sequence that comprises fewer than 35 amino acid residues. CPPs can cross the cellular membrane and mediate the uptake of cargo into cells, transporting therapeutically active cargoes, such as nucleic acids [4], proteins [5], imaging reagents [6], and small molecules [7]. Although CPP applications to delivery technology are fairly recent, the CPP itself was recognized through the 1988 discovery of the HIV transactivator of transcription protein (TAT) [8], along with evidence that the CPP could translocate through cell membranes. After the initial discovery that the shortened TAT sequence conferred an ability to penetrate cellular membranes, research into CPPs evolved rapidly, and the number of synthetic CPPs increased dramatically. Synthetic CPPs originate from a variety of sources and have different sequences and physicochemical characteristics. Nevertheless, some important CPP characteristics are held in common, chief among them: (i) limited cytotoxicity; (ii) an ability to facilitate receptor-independent transport across cell membranes when linked to oligonucleotides or proteins; and (iii) an ability to cross the cell plasma membrane in both energy-independent processes and endocytosis.

CPP classification

More than 100 different CPPs exist, with tremendous variation in their sequences. Thus, many different approaches to the classification of these peptides exist, including origin, function, sequence, mechanism of uptake, and biomedical application. However, on the basis of their physical chemical properties, CPPs can be divided simply into three main classes: cationic, hydrophobic, and amphipathic.

Glossary

Activatable cell-penetrating peptide (ACPP): presentation of CPP through the biological characteristics of tumors, such as low pH or tumor-specific enzyme, or external stimuli, such as local hyperthermia.
Cell-penetrating peptide (CPP): peptides comprising 5–30 amino acids and most often arranged in positively charged sequences. This kind of peptide is noteworthy for its ability to cross cellular membranes.
Elastin-like polypeptide (ELP): a derivative of tropoelastin; comprises poly (Val-Pro-Gly-Xaa-Gly), wherein Xaa can be any amino acid except proline. It has a thermoresponsive property that allows reversible phase transition between solid and liquid phase according to surrounding temperature.
Enhanced permeability and retention (EPR): macromolecules tend to accumulate more and reside longer in tumor tissue than in normal tissues, due to its permeable and leaky histological structure.
Local hyperthermia: raising a local body temperature (e.g., of a tumor) from its normal physiological range near 37 °C to approximately 42 °C.
Matrix metalloproteinase (MMP): a family of zinc-dependent endopeptidases that degrade the ECM. Given that the ECM maintains the 3D structure of the tissue and organs, tumor cells should degrade ECM to metastasize to other organs by using these enzymes. MMP2/9 is categorized as a gelatinase.

Corresponding author: Raucher, D. (draucher@umc.edu).
Keywords: cell-penetrating peptide; anticancer; tumor targeting; elastin-like polypeptide.

1471-4914/
 http://dx.doi.org/10.1016/j.molmed.2015.06.005

Cationic CPPs

A commonly used cationic CPP contains highly positive net charges that originate from the basic short strands of arginines and lysines, both of which have a crucial role in mediating the internalization of various therapeutic cargoes. The two most widely used cationic CPPs are the Tat-derived peptide, Antennapedia homeodomain-derived peptide (Antp), and the nona-arginine peptide, which contains nine arginine amino acids [9,10].

Hydrophobic CPPs

A second class of CPPs is that of the hydrophobic peptides, which contain primarily nonpolar residues. These CPPs have hydrophobic amino acid groups that are crucial to cellular uptake, along with a low net charge. Hydrophobic CPPs include signal sequences from Kaposi fibroblast growth factor (K-FGF) [11] and fibroblast growth factor-12 (FGF-12) [12]. The addition of hydrophobic amino acids has been demonstrated to enhance the cellular uptake of short cationic peptides; nonpolar amino acids also comprise an essential part of amphipathic CPPs [13].

Amphipathic CPPs

Amphipathic peptides, which contain polar and nonpolar regions of amino acids, include peptides such as multiple antigen peptide (MAP) [14], integrin receptor targeting peptide arginine–glycine–aspartic (RGD) [15], and the herpes simplex virus protein VP22; all these peptides exhibit the property of intercellular transport and accumulate mainly in the nucleus [16]. The screening of natural proteins for protein transduction domains also continues [17]. However, an example of the steadily expanding search for other, more-efficient CPPs can be seen through the design of chimeric proteins, which contain domains isolated from different proteins. The hydrophobic fusion domain of the HIV-1 gp41 protein has been joined to the nuclear localization signal of the SV40 antigen (KKKRKV) to create the amphipathic CPPs MPG (with 27 residues: GALFLGFLGAAGSTMGAWSQPKKKRKV) and Pep-1 (with 21 residues: KETWWETWWTEWSQPKKKRKV) [18].

Mechanisms of CPP uptake

Although CPP intracellular uptake is not fully understood, several mechanisms that may enable CPP cellular entry exist, and may even coexist, depending on the physicochemical properties, concentration, charge, and length of the CPP. Cellular uptake further depends on the type, size, and charge of the cargo molecule, which would each yield differing efficiencies of accumulation and therapeutic applications [19]. However, the primary mechanisms for cellular uptake include the endocytosis-mediated pathway, the inverted micelle, and the carpet-like mechanism.

The endocytosis-mediated pathway

The endocytosis mechanism provides the major cellular uptake pathway for most CPPs. Although comprising two steps (endocytotic entry and endosomal escape), endocytosis may in fact involve several pathways. Among them are clathrin-mediated endocytosis [20], clathrin-independent endocytosis (such as endocytosis via lipid rafts or caveolae), and micropinocytosis [21,22].

Although various endocytotic mechanisms result in the penetration of CPPs into cells, problems concerning the escape of CPPs from the endosomes persist. As an example, TAT fused to a large molecule will remain primarily in the endosomes, while TAT attached to a small cargo will be distributed in the cytoplasm. These differing outcomes indicate that the type and size of CPP cargo exerts an influence on its uptake pathway and distribution [23].

The inverted micelle model

The inverted micelle model has been proposed as an attempt to explain translocation for the penetratin family of CPPs [24]. Penetratin CPPs are lysine- and arginine-rich peptides that can cross the cellular membrane at low temperatures, suggesting an energy-independent mechanism. Furthermore, the cellular uptake of penetratin CPPs is independent of any membrane receptors. The mechanism posited by the inverted micelle model holds that positively charged residues of the CPP (arginine and lysine) initially bind to the negatively charged phospholipids of the selectively permeable cell membrane [25]. Given that the CPP is thereby able to merge with this membrane, pocket-like micelles are formed that contain the CPP, which traverses the cell membrane within this micelle toward the cytoplasm. As these micelles cross the membrane, they invert, releasing the CPP and its cargo into the cells.

The carpet-like mechanism

The carpet-like mechanism, initially proposed to describe the penetration of desmaseptin [26], has now also been observed for other cationic antimicrobial CPPs [27]. These peptides have the ability to penetrate the cell membrane of microorganisms and cause cell lysis. According to the carpet-like model, the positively charged domain of these lytic peptides initially binds to negatively charged phospholipids on a cell surface, covering the cell membrane in a carpet-like manner. As a particular area of the membrane reaches a certain concentration of these peptides, the membrane becomes locally destabilized, leading to pore formation that enables passage of the CPPs and their cargo into the cells [28].

Increasing cell type specificity for CPP-mediated drug delivery

CPPs can now provide an effective means of intracellular drug delivery; however, they do not have cell type specificity. Given that the mechanism of CPP uptake involves a strong binding of CPPs to membrane lipids, most CPPs will be internalized by all cell types. This ubiquitous internalization currently represents a major obstruction to the clinical potential of CPPs as a method for selectively delivering highly active chemotherapeutics to cancer cells.

Despite some common properties, CPP families vary in their means of binding to the plasma membrane and also in their translocation mechanisms. Binding and translocation may depend on the extracellular matrix (ECM) components of the cellular plasma membrane, as well as on its lipids and sugar composition. Thus, a particular CPP may preferentially bind to cells whose membrane composition favors an interaction with the membrane components of