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Review

Recent progress of cell-penetrating peptides as new carriers for intracellular cargo delivery

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ABSTRACT

The plasma membrane as a selectively permeable barrier of living cells is essential to cell survival and function. In many cases, however, the efficient passage of exogenous bioactive molecules through the plasma membrane remains a major hurdle for intracellular delivery of cargoes. During the last two decades, the potential of peptides for drug delivery into cells has been highlighted by the discovery of numerous cell-penetrating peptides (CPPs). CPPs serving as carriers can successfully intracellular transport cargoes such as siRNA, nucleic acids, proteins, small molecule therapeutic agents, quantum dots and MRI contrast agents. This review mainly introduces recent advances of CPPs as new carriers for the development of cellular imaging, nuclear localization, pH-sensitive and thermally targeted delivery systems. In particular, we highlight the exploiting of the synergistic effects of targeting ligands and CPPs. What's more, the classification and cellular uptake mechanisms of CPPs are briefly discussed as well.

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1. Introduction

The plasma membrane as a selectively permeable barrier of living cells is essential to cell survival and function. Although small-molecule drugs can traverse this membrane via a natural cellular process or direct diffusion through the lipid bilayer and protein-based drugs can enter cells by membrane mobile transport, in many cases the efficient passage of exogenous bioactive molecules through the plasma membrane remains a major barrier for intracellular delivery of cargo. Therefore, molecular transporters have long been sought that would enhance the transportation efficiency of therapeutic and imaging agents into living cells.

Although a variety of vectors have been chosen as candidates for cargo translocation, cell-penetrating peptides (CPPs) are one of the most popular and efficient vectors for achieving cellular uptake. CPPs are a class of diverse peptides, typically with 5–30 amino acids, and unlike most peptides, they can cross the cellular membrane [1,2]. It has been twenty years since the discovery of the first CPP and the concept of protein transduction into cell presented in 1988 by Frankel and Green in parallel. They discovered that the transactivator of transcription (TAT) protein of HIV can cross cell membranes and be efficiently internalized by cells *in vitro*, which results in transactivation of the viral promoter [3]. These discoveries, along with those identifying other peptides with membrane-crossing activities, served as the cornerstone for a new subfield which focuses on the use of CPPs as molecular transporters. From then on, the list of available CPPs has grown rapidly and CPPs have been employed for a variety of applications [4]. CPPs serving as vectors can successfully intracellular transport cargoes such as siRNA [5–7] nucleic acids [8,9], small molecule therapeutic agents [10,11], proteins [12,13], quantum dots [14], and MRI contrast agents [15], both *in vitro* and *in vivo*. In addition, this efficient transport system has lower cytotoxicity in a variety of cell lines compared with other delivery methods [16].

CPPs have received attractive attention for medical applications, not only because of their high internalization ability but also due to their potential for variable modification design. The following parts illustrate the numerous examples where CPPs have been exploited as new carriers for biology and medicine application. It is also concerned in the classification and cellular uptake mechanisms of CPPs.

2. Classification of CPPs

As to the classification of CPPs, there are a lot of approaches. For example, CPPs can be divided into subgroups defined by their origin or sequence characteristics [3]. Except for this general CPP classification, additional CPP subgroups should be mentioned. Bipartite peptides, whose origin is chimeric, contain two or more of the listed motifs and include several CPPs, such as transportan, pVEC, MAP, and Pep-1. Another subgroup of CPPs is based on proline-rich and polyproline amphipathic sequences including the sweet arrow peptide (SAP) [17], which is a sequence with 50% proline content in addition to three arginine residues that is derived from a storage protein in maize. Lorents A et al. demonstrated that the uptake of various CPPs increases the intracellular Ca^{2+} levels in Jurkat and HeLa cells, indicating that CPPs can be split into two major classes. One is amphipathic CPPs, which modulate the plasma membrane integrity inducing influx of Ca^{2+} and activating downstream responses starting from low concentrations; another is non-amphipathic CPPs that do not evoke changes at relevant concentrations [18]. Hitherto, the data collected from publications and patents have presented more than 100 diverse CPPs (varying from 5 to 40 amino acids in length). Table 1 presents a broad overview of the current CPP landscape from their sequence, origin, function and biomedical applications. Even though CPPs have a great sequence variety, it is possible to be mainly divided into three subgroups defined by their physical–chemical properties: cationic, amphipathic and hydrophobic.

2.1. Cationic CPPs

Cationic peptides are a class of peptides with a high positive net charge and few acidic amino acid residues. Studies suggest that at least eight positive charges are needed for efficient uptake of several cationic CPPs [19]. Cationic CPPs were originally considered as 'Trojan horse' delivery vehicles that enter cells without eliciting a cellular response [20]. Nevertheless, cationic CPPs can induce a wide range of side effects, including effects on membrane integrity and cell viability, which might be more subtle than cell death. Typical, the cationic CPPs are including R9 [21], Tat [22], hLF [23] and (RXR)4 [24] and so on.

2.2. Amphipathic CPPs

Peptides, such as MPG [25], penetratin [26] and CADY [27] which contain both polar and nonpolar regions are defined as amphipathic peptides, whose amphiphilicity may evolve from their primary structure.

Some primary amphipathic CPPs are chimeric peptides obtained by covalently attaching a hydrophobic domain for efficient targeting to cell membranes to a NLS. For example, MPG (GALFLGWLGAAGSTM GAPKKKRKV) is based on the SV40 NLS PKKRKV, and the hydrophobic domain of MPG is derived from the fusion sequence of the HIV glycoprotein 41. In MPG the hydrophobic domain is separated from the NLS through a linker (WSQP). Several other primary amphipathic CPPs are fully derived from natural proteins, such as pVEC [28], ARF (1–22) [29], and BPrPr (1–28) [30].

Secondary amphipathic α-helical CPPs have a highly hydrophobic patch on one face, whereas the other face can be cationic, anionic, or polar. The amphipathic β-sheet CPPs are based on one hydrophobic and one hydrophilic stretch of amino acids exposed to the solvent, such as vT5 (DPKGDPKGVTVTVTVTVTGKGDPKPD) [31].

A particularly interesting class of CPPs is proline-rich peptides, which have been reported in diverse families that differ in sequence and structure. However, they all contain a proline pyrrolidine template [32]. Pro-rich amphipathic peptides appearing interesting self-assembly properties have been extensively studied by CD and TEM. Some Pro-rich amphipathic peptides are derived from the N-terminal domain of γ-zein, a maize storage protein developed from the family of linear Pro-rich peptides [33]. Several other Pro-rich CPPs including bactenecin-7 (Bac7) [34], have been reported, which are synthetically derivatized polyproline-helix based peptides [35] with various R-groups attached to the pyrrolidine ring.

2.3. Hydrophobic CPPs

Peptides which contain only apolar residues are considered as hydrophobic peptides, which include stapled peptides [36], prenylated peptides [37], and pepducins [38]. So far, only a few hydrophobic CPP sequences have been discovered such as the signal sequences from integrin β3 (VTVLALGALAGVGVG) and Kaposi fibroblast growth factor (AAVALLPAVLLALLAP) [39]. Hydrophobic amino acids are also integral to amphipathic CPPs, such as MAP [40], and to other longer chimeric CPPs containing additional cationic residues for enhancing uptake and delivery capacity [41].

3. Cellular uptake mechanisms of CPPs

Although numerous studies about the uptake mechanism of CPPs across the plasma membrane have been reported in recent years, the pathways through which CPPs enter the cells have not been absolutely resolved [42]. One of the reasons for the difficulty in elucidating these mechanisms, in many cases, may attribute to various properties of peptides, such as charge and molecule length. Moreover, CPPs can interact with multiple cell surface molecules, including membrane lipids and membrane-associated proteoglycans [9,43]. What has become evident