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Review

Cell penetrating peptides: A concise review with emphasis on biomedical applications

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ABSTRACT

Introduction: The biological membranes as natural permeable barriers are required for survival and function of living cells. However, these natural barriers could be a major obstacle for the efficient intracellular delivery of therapeutic agents.
Materials and methods: In recent two decades, the use of peptides as novel carriers for intracellular cargo delivery has been received more attention by introducing the cell penetrating peptides (CPPs). CPPs, protein transduction domains, are an attractive class of short peptide sequences which can translocate across the cell membrane.
Conclusion: Owing to the ability of CPPs to transport across cellular membrane, they can employ as an appropriate carrier for various cargos include nucleic acid, proteins, SiRNA, therapeutic agents, nanoparticles and so on. In this review, we describe the classifications of CPPs, their uptake mechanisms as well as biomedical applications of the CPPs.

1. Introduction

The presence of biological membrane is a natural selective barrier for entrance of therapeutic agents. In order to transport across biomembranes, a drug must be either highly lipophilic or very small. On the other hand, novel therapeutic methodologies include gene and protein therapy because of cell-impermeable nature also have restricted their practical applications due to the peptides and oligonucleotides [1–5]. The current approaches for delivery of macromolecules, such as viral vectors and membrane perturbation methods, can lead to high toxicity, immunogenicity as well as low delivery yield. In recent decades, the use of peptides as novel carriers for intracellular cargo delivery has been received more attention by introducing the cell penetrating peptides (CPPs) [6–12].

CPPs, also known as protein transduction domains (PTDs), are positively charged short peptides with 5–30 amino acids long that can penetrate into biological membrane and deliver a wide variety of cargos into cells. TAT and penetratin were first CPPs which derived from HIV-TAT and Antennapedia homeodomain, respectively [13]. CPPs have received extensive attention in recent decades due to their high transduction efficiency (internalization efficiency of CPPs into cellular membrane) and also low cytotoxicity. Owing to the ability of these peptide sequences to transport across cellular membrane, they found to be as a promising candidate for intracellular delivery. Indeed, attachment of cargo molecules to the CPP, results in penetration of the intact cargos and then internalization into cells [14,15]. Conjugation CPP to cargo molecules could occur in two ways: covalent and non-covalent binding. In covalent conjugation, cargo molecules attach to CPP via covalent bond in a time-consuming process. This method has a serious drawback when transporting various cargos because each type of cargo needs its own covalent conjugation. In second approach, CPPs through electrostatic interaction bind to cargo [16]. This method due to its high flexibility is suitable for a wide range of cargo delivery applications. Overall, CPPs can apply for diagnostic and therapeutic applications, such as the delivery of fluorescent or radioactive compounds for imaging, delivery of peptides and proteins for therapeutic application, delivery of molecules into induced pluripotent stem cells for directing differentiation and so on [17]. Overall, delivery efficiency of the CPPs may be depending on some parameters such size of complex of cargo-CPP, nature of CPP, the type of peptide sequence and so on. For an instance, the CPP-cargo complex should be smaller than 200 nm in order to achieve the optimum endocytic uptake [18]. Besides, amphiphilc CPPs and arginine-rich CPPs because of high electrostatic interaction with negatively charged cellular membrane can significantly internalize into biomembranes [19]. This review provides a broad sight of classification, uptake mechanisms as well as biomedical applications of CPPs.

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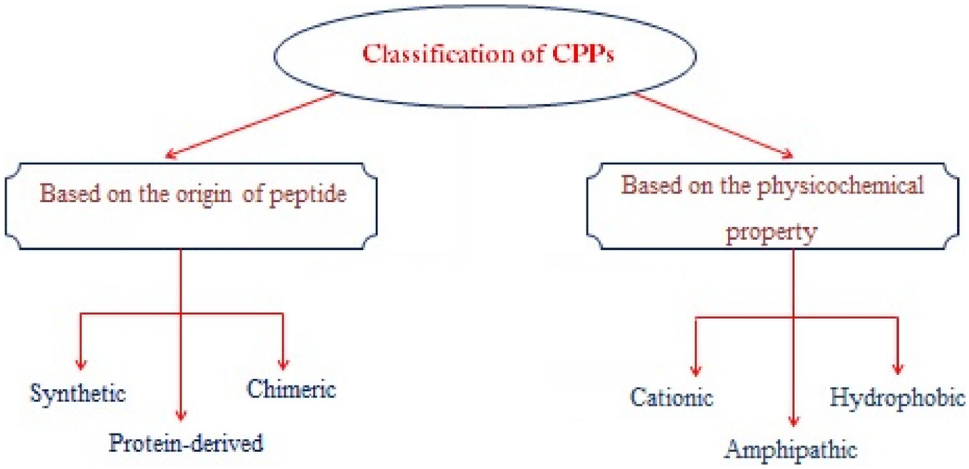

Fig. 1. Schematic diagram of various classifications of CPPs.

2. Classification of CPPs

In the literature, the unified classification has not been reported for CPPs; nevertheless these peptide sequences could be into two categories (Fig. 1) [20]:

1) Based on the origin of peptides.
2) Based on the physicochemical properties.

2.1. CPP classification based on the origin of peptides

Based on the origin of peptides, CPPs are divided into chimeric, protein-derived and synthetic. Chimeric CPPs are composed of two or more motifs from dissimilar peptides. Transportan is a chimeric CPP, which derived from galanin and mastoparan. TAT and penetratin that derived from natural proteins are examples of protein-derived CPPs. The polyarginine family, the simplest CPP mimics with argenin as the only structural component, belongs to synthetic peptides [21,22].

2.2. CPP classification based on the physicochemical properties

CPPs could be also categorized based on the physicochemical properties into three classes: Cationic, amphipathic and hydrophobic. Due to their positive charge, most of CPPs are cationic. This sub-class contains groups of polyarginine in their primary sequence. TAT, transcriptional activator protein in HIV-1, is an example of cationic CPP, which involves arginine and lysine residues [23]. Amphipathic CPPs are the sequences with a high degree of amphipathicity because of lysine residues in their structures. Transportan (a 27 amino acid-long peptide) is an amphipathic CPP [24]. Hydrophobic CPPs contain only hydrophobic motif/non-polar sequences. Generally, there are a few reports for the use of these CPPs as carriers compared to cationic and amphipathic CPPs [20]. Some examples of these two categories, accompanied by their sequences are summarized in Table 1.

3. Internalization mechanisms of CPP

The exact mechanism of transport of CPP across biological membranes is still obscure but in literature, three possible main pathways have been reported for CPP internalization into membrane; the peptide concentration, peptide sequence and lipid components in each membrane are three efficient parameters for selection of one of the internalization pathways of CPPs into the cellular membranes [14,25,26]. Based on the peptide concentration, the uptake route of many cationic CPPs can vary. At higher concentrations, rapid cytosolic uptake is detected that direct penetration suggests for CPPs, whereas the dominant mechanism of uptake is endocytosis at lower concentrations of peptides [27,28]. Peptide sequence is another influential parameter in the uptake mechanism of CPPs. In this regard, it need to be considered that for arginine-rich CPPs like Tat and penetratin, local concentrations of these peptides in biomembrane might be enhanced because of the high positive charge of CPPs results from the presence of several lysines or arginines via electrostatic interactions [29,30]. Whilst, affecting parameters on the membrane transduction of amphipathic CPPs like MAPs are helical amphipathicity and a length of at least four complete helical turns; hence, the uptake mechanism of this type of CPPs could be differed from that of Tat and penetratin analogous [31]. However, the positive charge of CPPs is an indispensable parameter for transport of biomaterial across the cellular membrane. But it needs to be pointed out that charge alone is not enough for a description of uptake process. Moreover, some of the reports have been displayed that peptide-to-cell ratio can influence on the uptake mode. For an instance, in higher ratios of peptide-to-cell, direct penetration along with endocytosis pathway can be occurred [32]. The impact of lipid components have been also investigated; In the case of the ionic interaction of peptide sequences with positive charge and biomembranes with negative charge, heparin sulfate proteoglycans or phospholipids play a pivotal role [33,34]. Proteoglycans, the major component of the extracellular matrix, possess a crucial role in the regulation of cell surface microdomains and are evidence for the first contacts between the CPPs and the cell surface happen via electrostatic interaction with cell surface proteoglycans GlucosAminoGlycan (GAG) platform, follow by a remodeling of the actin network and a selective activation of the direct relations between cytoskeletal organization and activation of small GTPases [35,36]. Consequently, GTPase activation and actin remodelling establish the start of the internalization mechanism and then have an important influence on membrane fluidity, thereby promoting cell entry of CPPs [37]. Although, the effect of membrane constituents on the uptake mode event may differ for individual CPPs [38]. Fig. 2, illustrates three proposed mechanisms for translocation of CPPs with cargo into cells. Three possible mechanisms for internalization of CPPs include:

- Direct penetration
- Endocytosis pathway
- Translocation through the formation of a transitory structure

3.1. Direct penetration

The direct penetration is an energy-independent pathway, which include various mechanisms, e.g. pore formation [39], the carpet-like model [40] and the membrane thinning model [41]. The interaction of the positively charged CPP with negatively charged membrane