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Review

In vitro and *in vivo* delivery of therapeutic proteins using cell penetrating peptides

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ABSTRACT

The failure of proteins to penetrate mammalian cells or target tumor cells restricts their value as therapeutic tools in a variety of diseases such as cancers. Recently, protein transduction domains (PTDs) or cell penetrating peptides (CPPs) have been shown to promote the delivery of therapeutic proteins or peptides into live cells. The successful delivery of proteins mainly depends on their physicochemical properties. Although, linear cell penetrating peptides are one of the most effective delivery vehicles; but currently, cyclic CPPs has been developed to potently transport bioactive full-length proteins into cells. Up to now, several small protein transduction domains from viral proteins including Tat or VP22 could be fused to other peptides or proteins to entry them in various cell types at a dose-dependent approach. A major disadvantage of PTD-fusion proteins is primary uptake into endosomal vesicles leading to inefficient release of the fusion proteins into the cytosol. Recently, non-covalent complex formation (Chariot) between proteins and CPPs has attracted a special interest to overcome some delivery limitations (*e.g.*, toxicity). Many preclinical and clinical trials of CPP-based delivery are currently under evaluation. Generally, development of more efficient protein transduction domains would significantly increase the potency of protein therapeutics. Moreover, the synergistic or combined effects of CPPs with other delivery systems for protein/peptide drug delivery would promote their therapeutic effects in cancer and other diseases. In this review, we will describe the functions and implications of CPPs for delivering the therapeutic proteins or peptides in preclinical and clinical studies.

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1. Introduction

Therapeutic effects of proteins are limited by their low potency to cross cell membranes. Some proteins possess natural biological functions as well as the ability to penetrate mammalian cells. The studies showed that the number of positively charged amino acids will affect the potency of cell penetration. For example, cationic peptides with 8–15 positively charged amino acids showed high potency of cell penetration but the permeation was suppressed by additional positively charged amino acids [1]. As known, proteins differ in charge, size, and structure; thus these properties influence complex formation between the protein of interest and the delivery agent [2]. Generally, the effective protein delivery using different transfection agents (/carriers) depends on some factors such as: a) the formation of electrostatic and hydrophobic interactions between protein and carrier; b) the used volume of carrier for prevention of cell toxicity and optimization of protein delivery; c) the optimal amount of protein, and d) the cell type for internalization of the complexes and their release in the cytoplasm. In addition, protein delivery should be performed in the absence of serum, because serum dissociates the non-covalent complexes of protein: delivery agent [2]. Up to now, hundreds of cell penetrating peptides (CPPs) were known as biological delivery agents. These small peptides usually consist of less than 30 amino acids, derived from natural proteins or synthesized as biomolecule-internalizing vectors [3,4]. The CPPs can be divided into cationic, amphipathic and hydrophobic types according to the physicochemical properties [5]. However, some deviations in CPP-mediated delivery may occur due to various properties of cell lines or tissues such as the lipid composition or protein content of the cell membrane, and the rate of endocytosis [6]. Among CPPs, HIV-1 Tat peptide is the first known CPP that can efficiently deliver different cargoes into cells. The second identified peptide is penetratin that naturally enters nerve cells and regulates their morphogenesis [7,8]. However, enhancement of safety, efficacy, bioavailability, and reduction in toxic effects of CPPs are necessary for development of protein or peptide delivery [9]. For example, nasal co-administration of insulin and the typical CPP (penetratin) in rats led to 50% bioavailability and significant reduction of blood glucose as compared to administration of insulin alone [10]. In addition, a study showed the potential of oral administration of insulin and D-form of penetratin to facilitate intestinal uptake. Indeed, both D- and L-forms of penetratin decreased the degradation rate for insulin, but D-penetratin (rqikiwfqnrrmkwkk) indicated an increased resistance to enzymes in comparison with L-penetratin (RQIKIWFQNRRMKWKK) and led to the highest amount of bioavailability [11]. Recently, several abnormal cell penetrating peptides have attracted a special interest for intracellular delivery of therapeutic proteins [1]. Regarding to the importance of CPPs in pharmacological and medical sciences, we will briefly describe their types, mechanisms, and functions in protein or peptide delivery. Fig. 1 shows an overview of subjects about CPPs in this article.

2. Abnormal cell penetrating peptides (CPPs)

Up to now, several abnormal cell penetrating peptides (CPPs) were known to represent intracellular activity termed as naturally supercharged human proteins (NSHPs). Their physicochemical properties including charge, structure or surface area were different from cationic peptides. The NSHPs were able to internalize the fused proteins up to 40-fold higher than usual CPPs. For example, heparin-binding EGF-like growth factor (HBEGF), a 28-amino acid peptide from the bZIP domain of c-Jun bound to DNA, and a DEK nuclear protein involved in chromatin remodeling were used to deliver protein into mammalian cells. The studies showed that the N-terminal domain of DEK (N-DEK) could efficiently penetrate cells and escape endosomes *in vivo* [1]. Recently, the engineered