Peptides 57 (2014) 78–94

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Review

Cell penetrating peptides: Efficient vectors for delivery of nanoparticles, nanocarriers, therapeutic and diagnostic molecules

Samad Mussa Farkhani[a,e,*], Alireza Valizadeh[a], Hadi Karami[b], Samane Mohammadi[a], Nasrin Sohrabi[c], Fariba Badrzadeh[d]

[a] Department of Medical Nanotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Golgasht Street, 51664 Tabriz, Iran
[b] Department of Medical Biotechnology, Faculty of Medicine, Arak University of Medical Sciences, Sardasht, 38481 Arak, Iran
[c] Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Golgasht Street, 51664 Tabriz, Iran
[d] Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Golgasht Street, 51664 Tabriz, Iran
[e] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Article history:
Received 6 March 2014
Received in revised form 19 April 2014
Accepted 19 April 2014
Available online 30 April 2014

Keywords:
Cell penetrating peptides
Drug delivery
Endosomal escape
Nanocarrier
Nanoparticle
Gene delivery

ABSTRACT

Efficient delivery of therapeutic and diagnostic molecules to the cells and tissues is a difficult challenge. The cellular membrane is very effective in its role as a selectively permeable barrier. While it is essential for cell survival and function, also presents a major barrier for intracellular delivery of cargo such as therapeutic and diagnostic agents. In recent years, cell-penetrating peptides (CPPs), that are relatively short cationic and/or amphipathic peptides, received great attention as efficient cellular delivery vectors due to their intrinsic ability to enter cells and mediate uptake of a wide range of macromolecular cargo such as plasmid DNA (pDNA), small interfering RNA (siRNAs), drugs, and nanoparticulate pharmaceutical carriers. This review discusses the various uptake mechanisms of these peptides. Furthermore, we discuss recent advances in the use of CPP for the efficient delivery of nanoparticles, nanocarriers, DNA, siRNA, and anticancer drugs to the cells. In addition, we have been highlighting new results for improving endosomal escape of CPP-cargo molecules. Finally, pH-responsive and activable CPPs for tumor-targeting therapy have been described.

Contents

Abbreviations: ACPPs, activable CPPs; AE, active enediyne chromophore; alpha-GC, alpha-galactosylceramide; AoA, aminooxyacetic acid; APC, antigen presenting cell; ASCs, adipose tissue-derived stem cells; BBB, blood–brain barrier; β-CyD, β-cyclodextrin; β-gal, β-galactosidase; BLM, Bleomycin; CP-AuNPs, cyclic peptide-capped gold nanoparticles; Dox, doxorubicin; ELP, elastin-like polypeptide; EGFP, enhanced green fluorescent protein; FOL, folate; FITC, fluorescein isothiocyanate; GICs, glioma-initiating cells; PC, phosphatidylcholine; NLS, nuclear localization signaling; HA, hyaluronic acid; HAase, hyaluronidase; His, histidine; PNIPAm, Poly(*N*-isopropylacrylamide); LCST, lower critical solution temperature; R9, nona-arginine; R8, octa-arginine; SDC4, syndecan-4; SR9, synthetic nona-arginine; PE, phosphatidylethanolamine; PNA, peptide nucleic acid; MMP-2, matrix metalloproteinase-2; PLA, poly(lactic acid); SPIONs, superparamagnetic iron oxide nanoparticle; MT1-MMP, membrane type-1 matrix metalloproteinase; SV40, Simian virus 40; EGFR, epidermal growth factor receptor; VEGFR-1, vascular endothelial growth factor receptor-1; TF, tissue factor; siRNA, small interfering RNA; ND, neurodegenerative diseases; SCA1, spinocerebellar ataxia type 1; RVG, rabies virus glycoprotein; HRP, horseradish peroxidase; HA2, influenza virus hemagglutinin-2; MEND, multifunctional envelope-type nano device; ETA, exotoxin a; LMWP, low molecular weight protamine; PR, polyrotaxane; VCR, vincristine sulfate; PLD, pegylated liposomal doxorubicin; Glp, pyroglutamic acid; PEO, polyethylene oxide; PPO, polypropylene oxide; PPI, polypropylenimine; PAMAM, polyamidoamine; PEI, polyethylenimine; mAb 2C5, monoclonal antibody 2C5; MSNs, mesoporous silica nanoparticles; PTX, paclitaxel; PEG–DOPE, polyethylene glycol–dioleoyl phosphatidylethanolamine; WGA, wheat germ agglutinin.

* Corresponding author. Tel.: +98 411 3367914; fax: +98 411 2335950.
E-mail addresses: mosafarkhanis@gmail.com, mosafarkhanis@hotmail.com (S.M. Farkhani), a.valizadeh_medicalnano@yahoo.com (A. Valizadeh), Hadimolmed@gmail.com (H. Karami), asemane_m1986@yahoo.com (S. Mohammadi), Nasrin_sohrabi1986@yahoo.com (N. Sohrabi), badrzadehf@yahoo.com (F. Badrzadeh).

http://dx.doi.org/10.1016/j.peptides.2014.04.015
0196-9781/

1. Introduction

The plasma membrane of the cells is very effective in its role as a selectively permeable barrier. While this phospholipid bilayer is essential for cell survival and function, it also presents a major challenge for intracellular delivery of cargo such as therapeutic and diagnostic agents [47,189]. Efficient delivery of these molecules to the cells and tissues is a difficult challenge. Therapeutic agents with low cells membrane permeability are commonly considered to be of limited therapeutic value. An even more difficult task is to deliver hydrophilic molecules across the blood–brain barrier (BBB), a dynamic interface that prevents transport of most drugs from the vasculature into the brain parenchyma [136,137]. Thus, there is a growing effort to improve the cellular uptake of these compounds [173]. Over the past few decades, several medicinal carriers, such as polymers, nanospheres, nanocapsules, liposomes, micelles, and dendrimers have been widely used to deliver therapeutic and diagnostic agents to the cells. Because delivery of these nanocarriers is generally based on their passive accumulation in the pathological regions, they cannot efficiently deliver their therapeutic and diagnostic cargo to specific cells or to particular intracellular components [79]. In recent years, small peptides, that are relatively short cationic and/or amphipathic peptides, received attention as cellular delivery vectors due to their intrinsic ability to enter cells and mediate uptake of a wide range of macromolecular cargo [86]. These peptides typically with 5–30 amino acids, are usually known as cell-penetrating peptides (CPPs), membrane translocation sequences, "Trojan peptides" or protein transduction domains [75]. The first CPPs discovered in 1988, that were sequences from HIV-1 encoded TAT protein, TAT(48–60), and delivered very efficiently through cell membranes of cultured mammalian cells [54]. Afterwards, additional polycationic peptides of natural VP22, AntP and synthetic origin (transportan) have been identified which also facilitate cellular uptake, alone or together with attached cargoes such as genes, drugs or even nanoparticles (NPs) [23,36]. Since then, many novels synthetic or chimeric CPPs have been found and tested (Table 1). There are three basic types of CPPs that have been utilized for intracellular delivery of therapeutic molecules. Cationic CPPs, which are the first discovered CPP and derived from the HIV-1 protein TAT [53]. These peptides are short amino acid sequences that are often consists of arginine, lysine and histidine. Arginine has a guanidine head group that can form hydrogen bonds with the negatively charged phosphates and sulfates on the cell membrane and might lead to internalization with cell surfaces under physiological pH conditions. The charge of lysine is positive similar to arginine, but it does not contain the guanidine head group, and therefore is less effective at penetrating to the plasma membrane when acting alone. These cationic amino acids are mediated the interaction of the peptide with anionic/acidic motifs on the cell membrane in a receptor-independent fashion [23,79]. Studies suggest that at least eight positive charges are needed for efficient cellular uptake of cationic CPPs [50]. Hydrophobic peptides, that they contain hydrophobic amino acids and have a low net charge. Amphipathic CPPs, which divided into primary amphipathic CPPs (e.g. Pep-1, pVEC), secondary amphipathic α-helical CPPs (e.g. hCT18–32), β-sheet amphipathic CPPs (e.g. VT5), and Proline-rich amphipathic CPPs (e.g. Bac7) [115]. These peptides have lipophilic and hydrophilic blocks that are responsible for mediating the peptide translocation across the cell membrane [23]. CPPs can overcome the poor permeability of biological molecules through the plasma membrane of cells via adsorption to glycosaminoglycans of the cell membrane and processing through the endocytic mechanism [72,74,125], especially macropinocytosis [45,168]. The range of CPP-compatible cargo molecules is wide and it can act as vectors for siRNA, nucleic acids, small molecules, proteins, cytotoxic drugs and imaging contrast agents both in vitro and in vivo [58,115]. One of the advantages of using CPPs for therapeutic and diagnostic delivery into the cells is the lack of toxicity in comparison to the other carriers, such as liposomes, polymers, etc. However, one of the problems using exogenous peptides for delivery of various molecules to mammalian cells is the ability to induce a humoral immune response against the therapy, which can be highly harmful for the subsequent treatments. In this review we discuss the mechanisms of peptide uptake. We also highlight the recently uses of CPPs in delivery of nanoparticles, nanocarriers, genes and anticancer agents into mammalian cells. Additionally, we have been highlighting new results for improving endosomal escape of CPP-cargo molecules. Finally we discuss use of CPPs on the surface of nanocarriers for tumor-targeted anticancer drug delivery.

2. Mechanisms of CPP uptake

Understanding the uptake mechanism and intracellular trafficking of drug carriers is an essential step for optimization of these systems to produce maximum effect, and to determine the intracellular behavior and efficiency of the cargo delivery [75]. Though CPPs have been widely used to deliver cargo molecules into cells, the exact uptake mechanism of these peptides is still challenging lots of questions and it is not yet known whether cellular entry of CPPs happens with or without the mediation of specific cellular receptors [24]. Nevertheless, the two major cellular uptake mechanisms of CPP include nonendocytotic or energy-independent pathways and the endocytotic pathways have been proposed depending on CPP own features, the carried molecule, the cell type and the membrane lipid composition [175]. The different uptake mechanisms proposed to explain the internalization of free or cargo-conjugated CPPs is shown in Fig. 1. CPPs with small cargoes, may enter cells quickly via direct translocation in addition to the endocytic way. Uptake of large molecules attached to these peptides tended to be mediated by macropinocytosis in an energy-dependent manner with slower rates for larger compounds [106].