Applications of Cell-Penetrating Peptides in Tumor-Targeted Therapy and Disease Diagnosis (Postprint)

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Abstract

In recent years, cell-penetrating peptides (CPPs) have been widely applied in the biomedical field, providing an effective strategy for intracellular delivery of biomolecules. This review focuses on the role of CPPs in tumor therapy and disease diagnosis, with emphasis on their applications and advantages in tumor-targeted therapy and medical imaging diagnosis. Meanwhile, based on the characteristics of CPPs in drug delivery systems, addressing their existing limitations and expanding the possibilities for combination therapy have also become active areas of CPP research. This article provides a review of CPPs and their applications in the diagnosis and treatment of diseases such as tumors, and briefly describes optimization and improvement strategies, with the aim of promoting the clinical application of CPPs.

Full Text

Application of Cell-Penetrating Peptides in Tumor Targeted Therapy and Disease Diagnosis

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Abstract

In recent years, cell-penetrating peptides (CPPs) have been widely applied in the biomedical field, providing an effective strategy for intracellular delivery of biomolecules. This review focuses on the roles of CPPs in cancer treatment and disease diagnosis, emphasizing their applications and advantages in tumor

targeted therapy and medical imaging diagnosis. Meanwhile, improving upon the limitations of CPPs based on their characteristics in drug delivery systems and expanding their potential for combination therapy have become active research areas. We summarize CPPs and their applications in the diagnosis and treatment of tumors and other diseases, and briefly describe optimization and improvement strategies to facilitate the clinical translation of CPPs.

Keywords: cell-penetrating peptides; tumor; CPP

Cell-penetrating peptides (CPPs), also known as protein transduction domains or Trojan horse peptides, represent a large family of short amino acid sequences (5-30 residues) capable of crossing biological membranes and delivering diverse cargo molecules into cells, including small molecules, nucleic acids, proteins, viruses, imaging agents, and drugs [1, 2].

In 1988, two research groups independently discovered the first protein capable of crossing cell membranes [3, 4]. They observed that the human immunodeficiency virus-1 (HIV-1) trans-activator protein (Tat) could enter cultured cells, translocate to the nucleus, and transactivate viral gene expression. Studies revealed that the -helical domain of Tat protein, composed primarily of basic amino acids, serves as the main determinant for cellular internalization and nuclear translocation [5]. The Tat dodecapeptide GRKKRRQRRRPQ has been identified as the minimal functional unit, with many CPPs derived from this original sequence [6]. In 1991, the homeodomain encoded by the *Drosophila* Antennapedia gene was shown to cross neuronal membranes, translocate to the nucleus, and induce neuronal morphological differentiation [7]. The 16-amino acid peptide derived from the third helix of the Antennapedia homeodomain (RQIKIWFQNRRMKWKK), defined as penetratin, can efficiently cross cell membranes in an energy-independent manner [8].

Over the past three decades, CPPs have been extensively utilized in basic research and preclinical studies for treating various diseases including infections, inflammation, neurodegenerative disorders, and cancer [9]. However, due to limited plasma membrane permeability, low delivery efficiency, and poor specificity for target tumor cells, only a subset of CPPs have been evaluated in clinical trials. Current research employs diverse experimental approaches to develop CPPs that can specifically reach cancer cells and deliver therapeutic agents into their interior.

2. Classification of CPPs

Currently, over 100 CPPs exist , exhibiting substantial sequence diversity. These CPPs can be classified using different criteria, such as origin, function, sequence, uptake mechanism, and biomedical application. Based on their physicochemical properties, CPPs can be divided into three major categories: cationic CPPs, hydrophobic CPPs, and amphipathic CPPs.

2.1 Cationic CPPs

Commonly used cationic CPPs contain abundant positive charges, derived from basic short chains of arginine (Arg) and lysine (Lys), both of which play crucial roles in mediating the internalization of various therapeutic agents. The two most widely used cationic CPPs are Tat-derived peptides such as Antennapedia homeodomain-derived peptides and polyarginine peptides containing nine arginine residues (Polyarginines) [11, 12].

Table 1: Commonly used cell-penetrating peptides

CPP Name	Amino Acid Sequence
Polyarginines	RRRRRRRRR (R9)
Tat49-57	RKKRRQRRR
Penetratin (Antennapedia)	RQIKIWFQNRRMKWKK
Pep-1	KETWWETWWTEWSQPKKKRKV

2.2 Hydrophobic CPPs

The second category comprises hydrophobic peptides containing primarily nonpolar residues. These CPPs possess hydrophobic amino acids that play important roles in maintaining low charge and facilitating cellular uptake. Hydrophobic CPPs include signal sequences derived from Kaposi fibroblast growth factor (K-FGF) [13] and fibroblast growth factor-12 (FGF-12) [14].

2.3 Amphipathic CPPs

The final category includes amphipathic peptides containing both polar and nonpolar amino acid regions, such as model amphipathic peptide (MAP) [15], integrin receptor-targeting peptide Arg-Gly-Asp (RGD) [16], and herpes simplex virus protein VP22. These peptides exhibit intercellular transport capabilities and primarily accumulate in the nucleus [17]. Screening of natural proteins for protein transduction domains is also ongoing [18]. Several CPPs can form noncovalent complexes with biomolecules and improve their delivery in mammalian cells. For example, linking the hydrophobic fusion domain of HIV-1 gp41 protein with the nuclear localization signal of SV40 large T antigen (KKKRKV) generates the amphipathic CPP MPG (GALFLGFLGAAGSTMGAWSQPKKKRKV). Similarly, Pep-1 (KETWWETWWTEWSQPKKKRKV), MPG, and Pep-1 form stable complexes with their respective cargo (oligonucleotides or proteins/peptides) through noncovalent electrostatic and hydrophobic interactions, facilitating CPP localization [19].

3. Uptake Mechanisms of CPPs

Although the cellular uptake mechanisms of CPPs remain incompletely understood, based on their distinct physicochemical properties, we hypothesize that

CPPs may enter cells through several independent mechanisms or a combination thereof. Meanwhile, cellular uptake of CPPs also depends on cargo type, size, and charge, resulting in varying accumulation and application efficiencies [20]. Currently accepted primary mechanisms of cellular uptake include endocytosis-mediated pathways, the inverted micelle model, and the carpet-like mechanism.

3.1 Endocytosis-Mediated Pathways

Endocytosis provides the major cellular uptake route for most CPPs. This process generally involves two steps: endosomal entry and endosomal escape. However, endosomal entry may actually involve several pathways, including clathrin-mediated endocytosis [21], clathrin-independent endocytosis (such as via lipid rafts or caveolae), and macropinocytosis [22, 23].

3.2 Inverted Micelle Model

Researchers have proposed the inverted micelle model to explain the translocation of CPPs across membranes [24]. Cell-penetrating peptides are lysine- and arginine-rich peptides that can cross cell membranes at low temperatures, indicating an energy-independent process. Furthermore, CPP uptake occurs independently of any membrane receptors. The inverted micelle model suggests that positively charged residues (arginine and lysine) initially bind to negatively charged phospholipids on the cell membrane surface [25], enabling membrane fusion and forming pocket-like micelles containing CPPs. Subsequently, CPPs cross the cell membrane within these micelles toward the cytoplasmic side. After traversing the membrane, these micelles invert, releasing the CPP and its cargo into the cell.

3.3 Carpet-Like Mechanism

The carpet-like mechanism was originally described to explain the permeation phenomenon of dermaseptin, an antimicrobial peptide from frog skin, and can also account for the cytotoxicity of CPPs at high concentrations. These peptides possess the ability to penetrate microbial cell membranes and cause cell lysis. According to the carpet model, the positively charged structural domains of these lytic peptides initially bind to negatively charged phospholipids on the cell surface, covering the membrane in a carpet-like fashion. When these peptides reach a certain concentration in specific membrane regions, the membrane becomes locally unstable, forming pores through which CPPs and their cargo can pass into cells [27].

4. Applications of CPPs in Tumor Therapy and Disease Diagnosis

Cancer represents a leading cause of human mortality, with chemotherapy being the most common treatment modality [28]. However, chemotherapy suffers from

numerous drawbacks, including poor drug penetration into tumor tissue, drug resistance resulting from high-dose or long-term treatment, and dose-dependent side effects. Poor drug penetration into tumor tissue primarily stems from dense connective tissue matrices on tumor cells that hinder molecular entry, as well as high interstitial pressure caused by abnormal blood and lymphatic vessels. CPPs can promote drug delivery by facilitating extravasation of cancer cells and drug penetration into tumor cells without affecting normal tissues. Since certain therapeutic antibodies or peptides can specifically recognize tumor cell targets, this strategy can enrich a certain amount of anticancer drugs in tumor tissue. Consequently, when drugs are localized and preferentially accumulate at tumor sites, their tumor-killing activity is greatly enhanced while toxicity to healthy tissues is reduced [29]. Endogenous stimuli such as activation of specific enzymes can enhance cell specificity, while external stimuli such as mild hyperthermia can promote cargo transport and increase accumulation of CPP-delivered drugs.

4.1 CPP-Mediated Delivery of Bioactive Molecules

Proteins, nucleic acids, or other small molecules can modulate cellular functions and produce therapeutic effects. However, due to the selective permeability of cells, these molecules exhibit significantly weaker activity in vivo compared to in vitro experiments. Typically, this results from their large molecular weight or anionic nature. CPPs provide one of the most important functions by delivering various compounds into the cytosol while maintaining biological activity.

4.1.1 Small Molecule Delivery Many chemotherapeutic small molecules, such as paclitaxel [30], cyclosporine A [31], and methotrexate [32], retain their activity when conjugated with CPPs. Notably, although CPP-drug conjugates may exhibit low activity in purified biochemical systems in vitro, efficient cellular uptake can compensate for this deficiency. Studies have demonstrated that despite a 20-fold potency loss compared to the free drug, CPP-methotrexate conjugates remain highly cytotoxic against methotrexate-resistant cell lines [32]. Because CPP conjugates can effectively increase the level of bioactive small molecules entering cells, they can offset the reduced drug activity.

4.1.2 Peptide and Protein Delivery Cells often limit the accumulation of bioactive macromolecules. However, using CPPs as molecular transporters allows these molecules to be delivered at sufficient intracellular levels to produce biological effects. Research has revealed the mechanism by which CPPs alter transcription factor activity, involving a STAT-6 peptide inhibitor. STAT-6 can bind phosphorylated proteins to prevent dimerization and inhibit activity [33]. For intracellular delivery, the inhibitory peptide was conjugated with the HIV-Tat-derived PTD4 CPP. The conjugate exhibited significant negative effects upon internalization and, when delivered to the upper respiratory tract of mice, attenuated ovalbumin-induced inflammatory responses and mucus production. This suggests that STAT-6 activity is associated with allergic diseases [34], and

STAT-6 inhibitory peptides hold promise as novel treatments for allergic rhinitis and asthma.

An example of CPP-mediated modulation of immune responses involves overcoming bacterial sepsis. Sepsis results from apoptotic loss of lymphocytes and dendritic cells, which suppresses immunity and ultimately leads to death [35, 36]. Since anti-apoptotic therapies have proven effective in improving survival rates [37], CPPs can treat sepsis by delivering anti-apoptotic proteins [38]. Bcl-xL and its BH4 domain were conjugated with Tat and administered in mice with sepsis-induced lymphocyte apoptosis. The study found that mice receiving the conjugates showed significantly reduced sepsis-induced lymphocyte apoptosis. This work represents the first use of CPPs to deliver anti-apoptotic proteins against sepsis-induced apoptosis. The system' s efficacy demonstrates a highly effective method for reducing post-sepsis immune system depletion and improving survival.

4.1.3 Nucleic Acid and siRNA Delivery Delivery of anionic biomolecules such as nucleic acids presents significant challenges. Nucleic acid uptake without assistance occurs only at very low levels, and vector-assisted delivery often leads to endosomal entrapment or degradation by nucleases [39, 40]. Two approaches have been widely adopted to circumvent these obstacles: engineering endosomal escape [41] and utilizing neutral nucleic acid analogs such as peptide nucleic acids (PNA) [42]. The latter are nucleobase derivatives with a peptide backbone that confer electrical neutrality and resistance to cellular degradation. Despite this modification, PNAs retain the complementary base-pairing function of natural nucleic acids.

CPPs also facilitate delivery of small interfering RNAs (siRNAs) for gene expression modulation [43]. Previous reports highlighted the effectiveness of Tat in delivering siRNA for gene silencing [44]. Recently, a novel CPP called "Peptide for Ocular Delivery" (POD) was found to deliver small molecules into ocular tissues [45]. POD successfully delivered siRNA into human embryonic retinal cells, observing >50% gene silencing effect. POD also delivered plasmid DNA into cells, achieving >50% transgene expression. Furthermore, topical administration of POD-dye conjugates to mouse corneas enabled effective distribution of delivered molecules to neighboring ocular tissues and enhanced cellular uptake rates. This work demonstrated the potential of CPPs to enhance nucleic acid delivery to ocular tissues. However, further research is ongoing to determine whether topically applied POD-nucleic acid conjugates to the cornea can also achieve ocular tissue distribution and accelerated cellular uptake.

4.2 CPPs in Imaging Applications

Currently, using CPPs to deliver imaging agents and biosensors represents a research focus [46-48]. Visualizing internal features and physiological structures in living organisms is crucial for understanding, diagnosing, and treating diseases. For example, direct observation of diseased tissues during surgery and

distinguishing pre-disease states in patients would have profound medical implications. In biomedical research, observing stem cell differentiation processes or tracking immune cell dynamics in animals facilitates fundamental understanding of these biological processes. Non-invasive biomedical imaging techniques such as fluorescence imaging and magnetic resonance imaging (MRI) have been applied for in vivo tissue visualization. However, due to poor penetration of some imaging agents into cells or tissues, inadequate cellular uptake, insufficient targeting, and lack of photostability of fluorophores, CPPs have been considered as delivery vehicles to optimize in vivo fluorescence imaging when conjugated with imaging labels.

Multiple fluorophore-CPP conjugates with enhanced stability and uptake have been developed as imaging agents. For example, silica nanoparticles doped with fluorescein and modified with Tat peptides can efficiently cross the blood-brain barrier to label neuronal tissues in rats [47]. Another example of CPP application in imaging was reported by Tsien and colleagues [49], who designed fluorescent peptide hairpins consisting of polyarginine peptides covalently linked to polyanionic fragments. The cellular uptake process induced by cationic peptides was blocked by a short acidic residue segment attached via a cleavable linker. Once the linker is cleaved by proteases, the inhibitory effect is released, allowing the cationic CPP to freely deliver its carrier into cells. Since targeted proteases are overexpressed in cancer cells, this approach can be used for selective tumor labeling.

The development of semiconductor nanocrystals or quantum dots (QDs) has provided a powerful alternative to molecular fluorophores for imaging applications. The size and composition of QDs lead to quantum confinement of electrons, endowing these materials with many advantageous optical properties [50]. Despite strong luminescence, photobleaching resistance, long luminescence lifetime, broad adsorption range, and narrow emission profiles, quantum dots are not always stable in biological environments and are poorly taken up by cells, hindering stable in vivo imaging. CPP technology can effectively address this challenge. Studies have shown that CdS:Mn/ZnS quantum dots functionalized with CPPs and injected into rat arteries can efficiently label brain tissue within minutes [47]. Tat-QDs not only cross the blood-brain barrier but can also be visualized with a handheld lamp, demonstrating significant potential for visualizing diseased tissues. Other CPPs such as Pep-1 and polyarginine [51, 52] can also deliver QDs into cells.

Although noncovalent delivery of quantum dots has been achieved, this is only true for Pep-1 [46]; all other CPPs appear to require chemical conjugation to enhance cellular uptake levels. CPPs can be linked to QDs in various ways: for example, through covalent bonds with cysteine and lysine residues [47], biotin conjugation to streptavidin-coated nanoparticles [52-54], or via polyhistidine peptide linkers with affinity for metals contained within nanocrystals [54].

5. Modification and Improvement of CPPs

CPPs currently provide effective means for intracellular drug delivery; however, they lack strong cell-type specificity. Since CPP uptake mechanisms involve strong binding to membrane lipids, most CPPs will be internalized by various cell types. This ubiquitous internalization represents a major obstacle to the clinical application of CPPs for selectively delivering highly active drugs to cancer cells [55].

Conjugating CPPs with homing peptides represents a strategy to enhance specific drug delivery to diseased tissues and cells. This approach combines the cell-penetrating ability of CPPs with the capacity of homing domains to recognize specific cell types. After homing domains are conjugated to CPPs, the specificity of CPP-based drug delivery systems increases [56]. Another common strategy for achieving selective drug delivery to cancer cells involves conjugating targeting ligands to CPPs. For example, when fused with Tat-derived CPPs, anti-Her-2/neu peptide mimetics targeting epidermal growth factor ErbB2 (overexpressed in 30% of breast cancers) successfully reduced nonspecific uptake and improved cancer cell-specific uptake efficiency [57]. Many studies have also focused on coupling CPPs with active targeting ligands such as RGD peptides, folate, or hyaluronic acid, which are expressed in tumors including tumor epithelial cells [58-60]. To enhance CPP advantages while improving cell recognition specificity, further research is needed to develop and validate effective in vivo strategies.

Although CPPs share some common characteristics, the CPP family differs in plasma membrane binding modes and translocation mechanisms. Binding and translocation may depend on extracellular matrix components (ECM) and lipid and sugar composition of the cell membrane [61]. Therefore, specific CPPs can preferentially bind to membrane components that facilitate interaction with target cells. For example, penetratin injected into mouse brains preferentially binds to -2,8-polysialic acid on neuronal cell surfaces and is selectively taken up by neurons [62]. In another study, Lim et al. [63] revealed specific binding of CPPs to cancer cells. The cell-penetrating motif of the anticancer peptide Buforin IIb can target cancer cells through specific interaction with gangliosides, followed by internalization via lipid-mediated macropinocytosis. These results demonstrate the tremendous potential of CPPs in drug delivery and underscore the need for research on CPP delivery of various chemotherapeutic agents to target cells in vivo.

From the perspective of modifying CPP conjugates, linkers can be designed with cleavable structures, such as acid-labile hydrazone bonds [64], reducible disulfide bonds [65], or protease-cleavable peptide bonds [66], to affect cargo release at tumor sites. However, such modifications cause slight structural changes to the cargo molecule or CPP, requiring confirmation that minor alterations do not significantly affect biological activity. When peptides serve as CPP cargo, the conjugation process is simpler; sequences encoding peptide cargo can be

genetically engineered into the N- or C-terminus of CPP sequences to generate fusion peptides. Orzechowska et al. [67] used this technique to design the TAT-BID construct, which sensitizes cancer cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), ultimately leading to apoptosis. If the cargo is a gene, the process is even simpler. Since most CPPs contain cationic amino acids, simple mixing can prepare complexes of CPPs with negatively charged nucleic acids (such as plasmids, miRNA, or siRNA). In Wang et al.' s study [68], a mixture of siRNA targeting polo-like kinase-1 (Plk-1) and R9 CPP in electrostatic complexes was delivered to MDA-MB-231 breast cancer cells both in vitro and in vivo, resulting in Plk-1 expression interference and slowed tumor growth.

Recent advances in genomics and proteomics have facilitated effective identification of molecular targets for different disease states [69]. Direct drug delivery to lesion sites is essential for successful drug development, requiring drug molecules to be cell-permeable. Crossing the plasma membrane poses a challenge for many therapeutic agents, with many drugs showing ideal activity in vitro but substantially reduced activity in vivo. Therefore, optimizing cellular delivery of therapeutic agents is paramount. Multiple studies have demonstrated that CPPs can improve the efficacy of several therapeutic agents by enhancing cellular uptake, offering advantages over other delivery vectors, including lower toxicity and more controlled effects. CPPs have successfully facilitated intracellular transport of therapeutic agents ranging from small molecules to large proteins or nucleic acids, indicating promising prospects for CPP-mediated drug delivery. Future efforts should establish a more comprehensive system for testing and evaluating CPP-therapeutic agent compounds for various diseases and conditions, applying this approach to more diseases and providing new insights for clinical therapy.

REFERENCES

[1] HEITZ F, MORRIS M C, DIVITA G. Twenty years cell-penetrating peptides: molecular mechanisms to therapeutics British journal of pharmacology, 2009, 157(2): 195-206.

[2] GUIDOTTI G, BRAMBILLA L, ROSSI D. Cell-Penetrating Peptides: from basic research clinics to trends in pharmaceutical sciences, 2017, 38(4): 406-24.

[3] FRANKEL A D, PABO C O. Cellular uptake of the tat protein from human immunodeficiency virus [J]. Cell, 1988, 55(6): 1189-93.

[4] GREEN M, LOEWENSTEIN P M. Autonomous functional domains of chemically synthesized human immunodeficiency virus tat trans-activator protein [J]. Cell, 1988, 55(6): 1179-88.

[5] RUBEN S, PERKINS A, PURCELL R, et al. Structural and functional characterization of human immunodeficiency virus tat protein [J]. Journal of virology, 1989, 63(1): 1-8.

[6] PARK J, RYU J, KIM K A, et al. Mutational analysis of a human immunodeficiency virus type 1 Tat protein transduction domain which is required for delivery of an exogenous protein into mammalian cells [J]. The Journal of general virology, 2002, 83(Pt 5): 1173-81.

[7] JOLIOT A, PERNELLE C, DEAGOSTINI-BAZIN H, et al. Antennapedia homeobox peptide regulates neural morphogenesis [J]. Proceedings of the National Academy of Sciences of the United States of America, 1991, 88(5): 1864-8.

[8] DEROSSI D, JOLIOT A H, CHASSAING G, et al. The third helix of the Antennapedia homeodomain translocates through biological membranes [J]. The Journal of biological chemistry, 1994, 269(14): 10444-50.

[9] VASCONCELOS L, PARN K, LANGEL U. Therapeutic potential of cell-penetrating peptides [J]. Therapeutic delivery, 2013, 4(5): 573-91.

[10] MARTIN M E, RICE K G. Peptide-guided gene delivery [J]. The AAPS journal, 2007, 9(1): E18-29.

[11] EL-SAYED A, FUTAKI S, HARASHIMA H. Delivery of macromolecules using arginine-rich cell-penetrating peptides: ways to overcome endosomal entrapment [J]. The AAPS journal, 2009, 11(1): 13-22.

[12] REGBERG J, SRIMANEE A, LANGEL U. Applications of cell-penetrating peptides for tumor targeting and future cancer therapies [J]. Pharmaceuticals, 2012, 5(9): 991-1007.

[13] DOKKA S, TOLEDO-VELASQUEZ D, SHI X, et al. Cellular delivery of oligonucleotides by synthetic import peptide carrier [J]. Pharmaceutical research, 1997, 14(12): 1750-5.

[14] NAKAYAMA F, YASUDA T, UMEDA S, et al. Fibroblast growth factor-12 (FGF12) translocation into intestinal epithelial cells is dependent on a novel cell-penetrating peptide domain: involvement of internalization in the in vivo role of exogenous FGF12 [J]. The Journal of biological chemistry, 2011, 286(29): 25823-33.

[15] FERNANDEZ-CARNEADO J, KOGAN M J, PUJALS S, et al. Amphipathic peptides and drug delivery [J]. Biopolymers, 2004, 76(2): 98-103.

[16] TIAN H, LIN L, CHEN J, et al. RGD targeting hyaluronic acid coating system for PEI-PBLG polycation carriers [J]. Journal of controlled release : official journal of the Controlled Release Society, 2011, 155(1): 47-55.

[17] ELLIOTT G, O' HARE P. Intercellular trafficking of VP22-GFP fusion proteins [J]. Gene therapy, 1999, 6(1): 149-51.

[18] DIETRICH U, DURR R, KOCH J. Peptides as drugs: from screening to application [J]. Current pharmaceutical biotechnology, 2013, 14(5): 501-12.

[19] MORRIS M C, DESHAYES S, HEITZ F, et al. Cell-penetrating peptides: from molecular mechanisms to therapeutics [J]. Biology of the cell, 2008, 100(4):

201-17.

[20] FONSECA S B, PEREIRA M P, KELLEY S O. Recent advances in the use of cell-penetrating peptides for medical and biological applications [J]. Advanced drug delivery reviews, 2009, 61(11): 953-64.

[21] RICHARD J P, MELIKOV K, VIVES E, et al. Cell-penetrating peptides. A reevaluation of the mechanism of cellular uptake [J]. The Journal of biological chemistry, 2003, 278(1): 585-90.

[22] FUTAKI S, NAKASE I, TADOKORO A, et al. Arginine-rich peptides and their internalization mechanisms [J]. Biochemical Society transactions, 2007, 35(Pt 4): 784-7.

[23] MAYOR S, PAGANO R E. Pathways of clathrin-independent endocytosis [J]. Nature reviews Molecular cell biology, 2007, 8(8): 603-12.

[24] MALER L. Solution NMR studies of cell-penetrating peptides in model membrane systems [J]. Advanced drug delivery reviews, 2013, 65(8): 1002-11.

[25] PROCHIANTZ A. Homeoprotein intercellular transfer, the hidden face of cell-penetrating peptides [J]. Methods in molecular biology, 2011, 683: 249-62.

[26] MOR A, NGUYEN V H, DELFOUR A, et al. Isolation, amino acid sequence, and synthesis of dermaseptin, a novel antimicrobial peptide of amphibian skin [J]. Biochemistry, 1991, 30(36): 8824-30.

[27] SHAI Y. Mode of action of membrane active antimicrobial peptides [J]. Biopolymers, 2002, 66(4): 236-48.

[28] FARKHANI S M, VALIZADEH A, KARAMI H, et al. Cell penetrating peptides: efficient vectors for delivery of nanoparticles, nanocarriers, and therapeutic and diagnostic molecules [J]. Peptides, 2014, 57: 78-94.

[29] RUOSLAHTI E. Tumor penetrating peptides for improved drug delivery [J]. Advanced drug delivery reviews, 2017, (110-111): 3-12.

[30] DESHAYES S, MORRIS M, HEITZ F, et al. Delivery of proteins and nucleic acids using a non-covalent peptide-based strategy [J]. Advanced drug delivery reviews, 2008, 60(4-5): 537-47.

[31] ROTHBARD J B, GARLINGTON S, LIN Q, et al. Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation [J]. Nature medicine, 2000, 6(11): 1253-7.

[32] LINDGREN M, ROSENTHAL-AIZMAN K, SAAR K, et al. Overcoming methotrexate resistance in breast cancer tumour cells by the use of a new cell-penetrating peptide [J]. Biochemical pharmacology, 2006, 71(4): 416-25.

[33] MCCUSKER C T, WANG Y, SHAN J, et al. Inhibition of experimental allergic airways disease by local application of a cell-penetrating dominant-negative STAT-6 peptide [J]. Journal of immunology, 2007, 179(4): 2556-64.

[34] TAMURA K, ARAKAWA H, SUZUKI M, et al. Novel dinucleotide repeat polymorphism in the first exon of the STAT-6 gene is associated with allergic diseases [J]. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 2001, 31(10): 1509-14.

[35] HOTCHKISS R S, SWANSON P E, FREEMAN B D, et al. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction [J]. Critical care medicine, 1999, 27(7): 1230-51.

[36] HOTCHKISS R S, TINSLEY K W, SWANSON P E, et al. Depletion of dendritic cells, but not macrophages, in patients with sepsis [J]. Journal of immunology, 2002, 168(5): 2493-500.

[37] HOTCHKISS R S, SWANSON P E, KNUDSON C M, et al. Overexpression of Bcl-2 in transgenic mice decreases apoptosis and improves survival in sepsis [J]. Journal of immunology, 1999, 162(7): 4148-56.

[38] HOTCHKISS R S, MCCONNELL K W, BULLOK K, et al. TAT-BH4 and TAT-Bcl-xL peptides protect against sepsis-induced lymphocyte apoptosis in vivo [J]. Journal of immunology, 2006, 176(9): 5471-7.

[39] PATIL S D, RHODES D G, BURGESS D J. DNA-based therapeutics and DNA delivery systems: a comprehensive review [J]. The AAPS journal, 2005, 7(1): E61-77.

[40] AKHTAR S, JULIANO R L. Cellular uptake of intracellular antisense oligonucleotides [J]. Trends in cell biology, 1992, 2(5): 139-44.

[41] LO S L, WANG S. An endosomolytic Tat peptide produced by incorporation of histidine and cysteine residues as a nonviral vector for DNA transfection [J]. Biomaterials, 2008, 29(15): 2408-14.

[42] RAY A, NORDEN B. Peptide nucleic acid (PNA): its medical and biotechnical applications and promise for the future [J]. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 2000, 14(9): 1041-60.

[43] MCMANUS M T, SHARP P A. Gene silencing in mammals by small interfering RNAs [J]. Nature reviews Genetics, 2002, 3(10): 737-47.

[44] CHIU Y L, ALI A, CHU C Y, et al. Visualizing a correlation between siRNA localization, cellular uptake, and RNAi in living cells [J]. Chemistry & biology, 2004, 11(8): 1165-75.

[45] JOHNSON L N, CASHMAN S M, KUMAR-SINGH R. Cell-penetrating peptide for enhanced delivery of nucleic acids and drugs to ocular tissues including retina and cornea [J]. Molecular therapy : the journal of the American Society of Gene Therapy, 2008, 16(1): 107-14.

[46] ROZENZHAK S M, KADAKIA M P, CASERTA T M, et al. Cellular internalization and targeting of semiconductor quantum dots [J]. Chemical communications, 2005, 17(17): 2217-9.

[47] SANTRA S, YANG H, DUTTA D, et al. TAT conjugated, FITC doped silica nanoparticles for bioimaging applications [J]. Chemical communications, 2004, 24(24): 2810-1.

[48] WEBSTER A, COMPTON S J, AYLOTT J W. Optical calcium sensors: development of a generic method for their introduction to the cell using conjugated cell penetrating peptides [J]. The Analyst, 2005, 130(2): 163-70.

[49] JIANG T, OLSON E S, NGUYEN Q T, et al. Tumor imaging by means of proteolytic activation of cell-penetrating peptides [J]. Proceedings of the National Academy of Sciences of the United States of America, 2004, 101(51): 17867-72.

[50] ALIVISATOS A P, JOHNSSON K P, PENG X, et al. Organization of 'nanocrystal molecules' using DNA [J]. Nature, 1996, 382(6592): 609-11.

[51] BALLOU B, LAGERHOLM B C, ERNST L A, et al. Noninvasive imaging of quantum dots in mice [J]. Bioconjugate chemistry, 2004, 15(1): 79-86.

[52] SILVER J, OU W. Photoactivation of quantum dot fluorescence following endocytosis [J]. Nano letters, 2005, 5(7): 1445-9.

[53] LAGERHOLM B C, WEINREB G E, JACOBSON K, et al. Detecting microdomains in intact cell membranes [J]. Annual review of physical chemistry, 2005, 56(1): 309-36.

[54] DELEHANTY J B, MEDINTZ I L, PONS T, et al. Self-assembled quantum dot-peptide bioconjugates for selective intracellular delivery [J]. Bioconjugate chemistry, 2006, 17(4): 920-7.

[55] YU W, ZHAN Y, XUE B, et al. Highly efficient cellular uptake of a cell-penetrating peptide (CPP) derived from the capsid protein of porcine circovirus type 2 [J]. The Journal of biological chemistry, 2018, 293(30): 11549-53.

[56] RUOSLAHTI E, BHATIA S N, SAILOR M J. Targeting of drugs and nanoparticles to tumors [J]. The Journal of cell biology, 2010, 188(6): 759-68.

[57] TAN M, LAN K H, YAO J, et al. Selective inhibition of ErbB2-overexpressing breast cancer cells by a novel TAT-based ErbB2-targeting signal transducers and activators of transcription 3-blocking peptide [J]. Cancer research, 2006, 66(7): 3764-72.

[58] SALAZAR M D, RATNAM M. The folate receptor: what does it promise in tissue-targeted therapeutics? [J]. Cancer metastasis reviews, 2007, 26(1): 141-52.

[59] KUNATH K, MERDAN T, HEGENER O, et al. Integrin targeting using RGD-PEI conjugates for in vitro gene transfer [J]. The journal of gene medicine, 2003, 5(7): 588-99.

[60] WANG L, SU W, LIU Z, et al. CD44 antibody-targeted liposomal nanoparticles for molecular imaging and therapy of hepatocellular carcinoma [J]. Bio-

materials, 2012, 33(20): 5107-14.

[61] XU Y, WANG B, KAUR R, et al. A Supramolecular [10]CPP Junction Enables Efficient Electron Transfer in Modular Porphyrin-[10]CPP superset-Fullerene Complexes [J]. Angewandte Chemie, 2018, 57(36): 11549-53.

[62] BOLTON S J, JONES D N, DARKER J G, et al. Cellular uptake and spread of the cell-permeable peptide penetratin in adult rat brain [J]. The European journal of neuroscience, 2000, 12(8): 2847-55.

[63] LIM K J, SUNG B H, SHIN J R, et al. A cancer specific cell-penetrating peptide, BR2, for the efficient delivery of an scFv into cancer cells [J]. PloS one, 2013, 8(6): e66084.

[64] LERICHE G, CHISHOLM L, WAGNER A. Cleavable linkers in chemical biology [J]. Bioorganic & medicinal chemistry, 2012, 20(2): 571-82.

[65] ALVES I D, CARRE M, MONTERO M P, et al. A proapoptotic peptide conjugated to penetratin selectively inhibits tumor cell growth [J]. Biochimica et biophysica acta, 2014, 1838(8): 2109-20.

[66] CHOI K Y, SWIERCZEWSKA M, LEE S, et al. Protease-activated drug development [J]. Theranostics, 2012, 2(2): 156-78.

[67] ORZECHOWSKA E J, KOZLOWSKA E, CZUBATY A, et al. Controlled delivery of BID protein fused with TAT peptide sensitizes cancer cells to apoptosis [J]. BMC cancer, 2014, 14: 14.

[68] WANG H X, YANG X Z, SUN C Y, et al. Matrix metalloproteinase 2-responsive micelle for siRNA delivery [J]. Biomaterials, 2014, 35(26): 7622-34.

[69] CHANG X, HOU Y. Expression of RecA and cell-penetrating peptide (CPP) fusion protein in bacteria and mammalian cells [J]. International journal of biochemistry and molecular biology, 2018, 9(1): 1-10.

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