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Review article

Cell-penetrating peptides as noninvasive transmembrane vectors for the development of novel multifunctional drug-delivery systems☆

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ABSTRACT

Unique characteristics, such as nontoxicity and rapid cellular internalization, allow the cell-penetrating peptides (CPPs) to transport hydrophilic macromolecules into cells, thus, enabling them to execute biological functions. However, some CPPs have limitations due to nonspecificity and easy proteolysis. To overcome such defects, the CPP amino acid sequence can be modified, replaced, and reconstructed for optimization. CPPs can also be used in combination with other drug vectors, fused with their preponderances to create novel multifunctional drug-delivery systems that increase the stability during blood circulation, and also develop novel preparations capable of targeted delivery, along with sustainable and controllable release. Further improvements in CPP structure can facilitate the penetration of macromolecules into diverse biomembrane structures, such as the blood brain barrier, gastroenteric mucosa, and skin dermis. The ability of CPP to act as transmembrane vectors improves the clinical application of some biomolecules to treat central nervous system diseases, increase oral bioavailability, and develop percutaneous-delivery dosage form.

Contents

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1. Introduction

Intercellular transposition of biomacromolecules is the basis of physiological and pathological processes. Cell-penetrating peptides (CPPs) act as cargo carriers and constitute a current hotspot in medical research. They are capable of entering the body in a noninvasive manner and accelerate the absorption of macromolecules *via* physiological mechanisms such as energy-dependent endocytosis and energy-independent direct penetration [1–4] (Fig. 1). Compared with some traditional techniques, such as microinjection and electroporation [5,6], CPPs do not destroy the integrity of the cell membranes, and are considered highly efficient and safe, thus, providing new avenues for research and applications in life sciences.

Through biomaterial modification, CPPs can be refolded and assembled with synthetic nanostructures in order to ameliorate the disadvantages owing to nonselectivity, lower delivery efficiency, and decreased susceptibility to degradation [7–9]. CPPs can be also incorporated into versatile cargo-carrying platforms to create novel drug-delivery systems that ensures improved coated-drug uptake, as well as their targeted recognition and controlled release *via* stimulus-responsive mechanisms [10,11]. The extensive use of CPPs in drug delivery will add considerable potency to protein- and nucleic-acid-based drugs [12–15], thereby increasing the possibility of biomacromolecular drugs crossing physiological barriers, such as the blood–brain barrier (BBB), nose mucous membrane, gastrointestinal mucosa, and skin.

2. Cell penetrating peptides (CPPs)

Currently, hundreds of CPPs have been found and used in biomedical research. CPPs are generally short peptides consisting of <30 amino acids and are divided into two main types as follows: 1) a polypeptide motif derived from natural proteins with penetrating functions, and 2) artificially designed and synthesized polypeptides, which are further optimized as molecule-internalizing vectors.

2.1. Natural-protein-derived CPPs

Many natural proteins can cross plasma membranes, including transactivators of gene transcription [16], DNA −/RNA-binding proteins [17], antimicrobial peptides [18,19], viral particle envelope proteins [20,21], plant circular skeletal proteins [22,23] and so on. The shortest amino acid sequences associated with these proteins and having penetration ability are determined to be CPPs, with several of these related to TAT [24], VP_{22} [25], Antp [26], gH_{625} [27,28], *etc.*

Studies found that CPPs have no cell specificity, and as the transport vehicle, can mediate effective internalization of exogenous proteins, plasmid DNA, antigens, fluorophores, and peptide nucleic acids into the cytoplasm and cell nucleus [29–33]. Furthermore, they have common properties, including rapid cell-membrane penetration, high levels of nuclear accumulation, identical entering efficiency between 4 °C and 37 °C, and low sensitivity to inhibitors of clathrin-mediated endocytosis [34].

2.2. Artificial CPPs

Some artificial CPPs have been designed and synthesized based on the structures of naturally-derived CPPs. These are fabricated *via* the replacement of key amino acids, splicing diverse functional sequences together, identification based on phage display, or screening by using messenger RNA-display technology [35,36]. These artificial CPPs are capable of being optimized for the improvement of their stability in circulating blood, to escape endolysosomal degradation, improve cellular internalization, and endow pH-responsive capabilities.

2.2.1. Amino acid replacement

There are two main amino acid substitutions. One is arginine accretion, and the other is histidine replacement. Arginine has a strong positive charge, which acts as a cell-penetrating motif. Their replacement can promote CPP penetrating capacity, as the number of arginines present in a sequence affects internalization efficiency. Various arginine-rich polypeptides, such as HIV-1 $Rev_{34–50}$, and FHV $coat_{35–49}$, contain transmembrane properties similar to $Tat_{48–60}$ [37]. Except for the presence of seven arginine residues, these polypeptides have no other similarities. Additionally, their internalization efficiency is attenuated as the number of arginine residue decreases, and is nearly lost when the number is <5 [38]. Amino acids of the penetratin protein are substituted for arginine or lysine, and the uptake efficiency has been compared between the mutant variants. It is found that penetratin-Arg exhibits higher penetration ability than that of penetratin-Lys [39]. SR_9, HR_9, and PR_9 are also arginine-replacing CPPs capable of transporting fluorescent protein into animal, plant, and bacterial cells [40,41]. These have been used to carry red quantum dots in the development of new types of bioimaging technologies [42,43], and transport DNA/siRNA into insect cells in order to improve their transfection efficiencies [44].

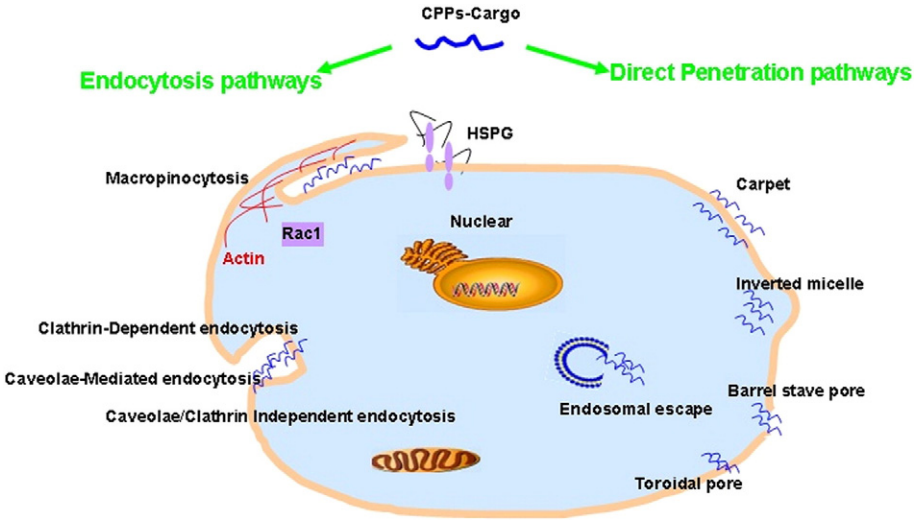

Fig. 1. Different internalized mechanism of CPPs-cargo. CPPs-cargo are internalized into the cell by means of the energy-dependent endocytosis or the energy-independent direct penetration. The endocytosis can be again divided into 4 kinds as follows: macropinocytosis, clathrin-dependent endocytosis, caveola-mediated endocytosis and caveola/clathrin independent endocytosis; and the direct penetration are also divided into 4 kinds as follows: carpet, inverted micelle, barrel-stave pore and toroidal pore.

Histidine can be used as a pH-responsive motif that has buffering capacity under physiological conditions (pKa 6.5) and endolysosome-escape ability (proton-sponge effect). Using histidine to substitute and modify CPP sequences results in novel pH-responsive CPPs. The mTAT (C-5H-TAT-5H-C) protein is synthesized by HIV-1 TAT and is covalently fused with ten histidine and two cysteine residues [45]. The histidine remains negatively charged under normal physiological conditions, but can be exchanged into positive-charge status under acidic conditions (pH 5–6), which endows TAT with endosomolytic ability. The mTAT/PEI shows significant improvements (up to 5-fold) in the transfection efficiencies of both the cell lines, with little cytotoxicity when compared with that of the four commercial reagents. H(7)K(R(2)) is designed as a tumor-specific pH-responsive peptide, which is constructed as a cell-penetrating motif [(R(2)) with seven histidines] [46]. At first, histidines form hydrophobic attractions, and the –(R(2)) moiety can be temporarily blocked onto the core of polymeric micelles. Secondly, the histidines are ionized, and the hydrophobic interaction is weakened in the acidic environment of the tumor tissue, followed by the –(R(2)) moiety being pushed out in order to increase cell internalization.

2.2.2. Functional motif chimera

To synthesize new CPPs, the conjugation of diverse functional amino acid sequences can be done according to electric charge and capacity. Pep-1 is an amphiphilic tandem peptide 21 amino acids in length and containing three domains: a hydrophobic tryptophan-rich region, a hydrophilic lysine-rich region, and a spacer-linker region for enhancing stability and flexibility [47]. This peptide can effectively enter HS-68 cells, and is not toxic to NIH-3T3, 293, or Jurkat cells when its concentration is < 100 μM. Pep-1 can mediate the entry of biomacromolecules into the cells and then be rapidly separated, eliminating the possibility of interfering with the localization and activity of the molecule [48,49]. The complex of Pep-1/cargo can be kept stable in physiological buffer solutions with insensitivity to serum.

Splicing disparate original CPP sequences can also be considered, allowing fabrication of CPPs with increased penetration capabilities. Transportan (TP) consists of 12 N-terminal amino acid residues from galanin and 14 amino acid residues of mastoparan, allowing retention of the partial capabilities of both galanin and mastoparan, whose internalization efficiency in plant cells is 2–3-fold higher than that of pVEC [50]. Besides, JB577 is designed as a modular cell penetrating peptide which mediates efficient endosomal escape of large protein, dendrimers and quantum dots (QDs) [51,52]. JB577 peptide consists of the sequence $WG\cdot(Dap^{Pal})\cdot VKIKK\cdot P_9\cdot GG\cdot H_6$, subdivided into disparate functional motifs, whose core is $(Dap^{Pal})\cdot VKIKK$. The His_6, Pro_9, Gly_2, Trp and Poly(L-proline) motifs are defined as the essential components required for the endosomal escape of JB577.

2.2.3. Screening from peptide libraries

Because some CPPs' internalization is nonselective, and their transduction efficiencies are also too low to be used as intracellular carriers for therapeutic purposes, some unrecorded encoding and special-function CPPs can be screened and identified from peptide libraries building on the phage-display and messenger RNA-display technology.

By fusion to one of the phage surface proteins, about 10^9 different peptides can be expressed on the phage surface, and then the desired peptides can be selected from these display peptides through binding to a particular molecule. Chen et al. applied Ph.D-C7C, a phage-display peptide library, onto the abdominal skin of BALB/cA nude mice and recovered phage particles from the blood circulation. Recovered phage was amplified and used for the next round of *in vivo* selection. Following these rounds, TD-1 peptide was identified, which was capable of permeating the intact skin [53]. Similarly, Whitney et al. used parallel *in vivo* and *in vitro* selection with phage display to identify some novel

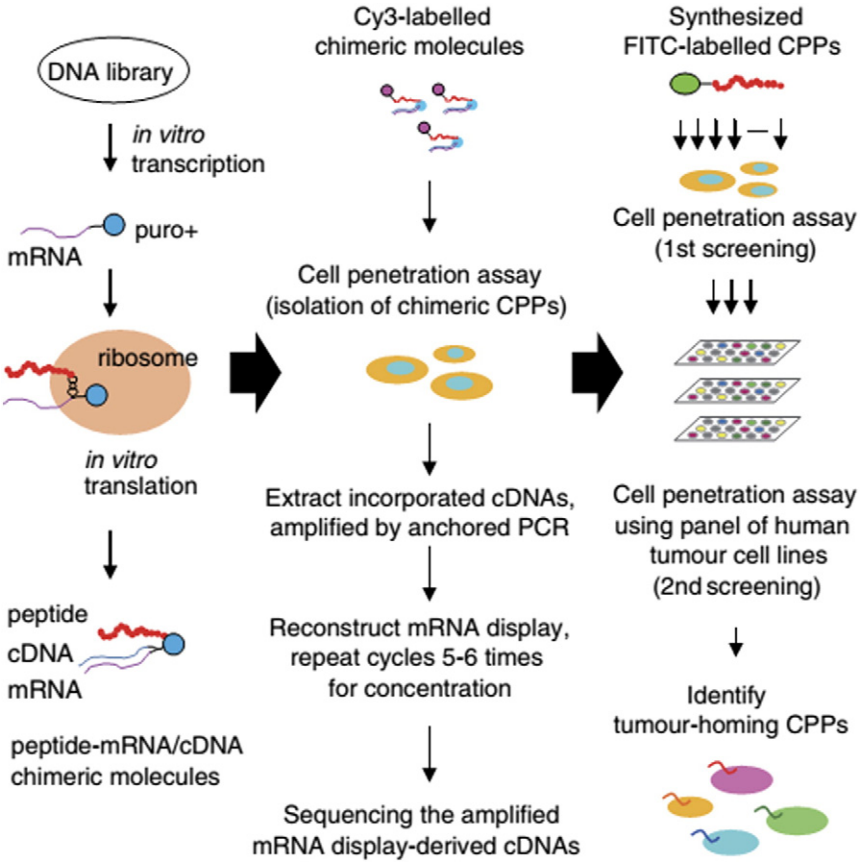

Fig. 2. Construction of mRNA display library and outline of screening method for isolation of tumor-homing CPPs. Reprinted with permission from ref. [56].

Table 1
A new multifunctional drug delivery system based on CPPs integrated with other vectors.

Delivery carrier	Special vector	CPPs	Purpose	Delivery system	Ref
Liposome	Cationic liposome	gh_{625}, MPS	Anti-cancer	PEG-gh_{625}-nanoliposome-MTX, MPS-cytC-liposome	[57] [58]
Polymers	PEI, PAMAM	Tat, polyArg	Gene therapy	Metal NPs@PEI-Tat, Bodipy-PAMAM-Tat-siRNA, R-PAMAM-PEG-PAMAM-R-DNA	[59] [60] [61]
Nanoparticles	Nanomicelles, MNPs, nanosilver, silica nanoparticles, nanogold	Tat, G_3R_6Tat, Tat_{49-57}	Anti-glioma, gene transfer, MRIA, antimicrobial anti-tumor PTA therapy	MPEG-PCL-Tat-nanomicelles, PEI-MNPs-Tat-DNA, AgNP-Tat, DOX-MSNs-Tat (99 m)Tc/(177)Lu-AuNP-Tat-BN, cpHDL-AuNRs,	[62] [63] [64] [65] [66] [67]

MRIA, magnetic resonance imaging applications; MNPs, magnetic nanoparticals; PTA, photothermal ablation; MSNs, mesoporous silica nanoparticles.

tumor-homing activatable cell penetrating peptides with no bias for primary sequence or target protease [54].

Compared with phage display, messenger RNA display technology is another method for the construction of random peptide libraries [55]. On account of the smaller molecular size of mRNA display-based complex and the larger number of peptides displayed, mRNA display is more tailored for isolating CPPs. Eisaku et al. applied this technology to gain ten novel CPPs as tumor lineage-homing CPPs, which could be possible to target specific tumor cells. The screening procedure is shown in Fig. 2. Among them, CPP_2 was successfully internalized by primary colon adenocarcinoma cells, and CPP_{44} was capable of invading hepatic tumor cells *in vitro*, and with specificity for myelogenous leukemia tumors *in vivo* [56].

3. Multifunctional drug-delivery systems (MDDSs) based on CPPs

The clinical application of CPPs for drug delivery is impeded due to their nonselectivity and weak stability. In addition to using modification, addition, or replacement of amino acid sequences to enhance self-integrity, CPPs can also be combined with other drug vectors, thus integrating with characteristics associated with various drug-transportation techniques in order to develop novel MDDSs (Table 1).

Through chemical attachment or modification of CPPs on the other drug vectors, such as liposomes, metal nanoparticles, and cation polymers, the MDDS can further increase the drug-loading, and enhance the biomembrane-crossing rates and the tissue-absorption efficiency of drugs. These activities can also decrease side effects of therapeutic agent, such as cellular toxicity, immunogenicity, and hemolytic activity, to strengthen therapeutic effects on diseases associated with tumors, inflammation, and viral infections.

3.1. Targeted drug delivery associated with CPPs

The glycosaminoglycans and phospholipids on tumor cell surfaces are negatively charged, enabling cationic CPPs to preferentially interact with and enter tumor cells. For example, penetratin can specifically bind to the chondroitin sulfate of tumor cellular membranes, increasing the anticancer properties of CPPs-cargo [68,69].

Due to the different components of the cell membrane between the tumor and the normal cells, it is possible to alter CPPs to preferentially target the tumor cells instead of the normal cells. However, this type of targeting using charged selectivity results in lower affinity and can impede the application of CPPs *in vivo* due to the biological milieu containing negatively charged serum proteins capable of attracting cationic CPPs [70]. Therefore, there is an urgent need to overcome the limitation of nonselectivity and indiscriminative distribution when CPPs are used in drug delivery *in vivo*.

Tumor cells express special receptors and markers, such as transferrin, RGD, Lys(3)-bombesin (BN), and NGR, which may constitute appropriate targets [71,72]. Incorporating with the characteristics of receptor-specific binding to a ligand, CPPs with these targets can be developed into the targeting preparations [73,74], which increases the capability of the CPP-complexed drug targeting to tumor tissue.

3.1.1. Combining with CPPs for the target drug delivery

Scientists synthesized tandem peptides after forming conjugations of CPPs with a ligand or antibody. These could be referred to tumor-homing peptides, endowing the CPPs with specificity toward tumor tissues [75]. Liu et al. combined R_8 with isomers of the Arg-Gly-Asp (RGD) peptide through an amide linkage to create three kinds of tandem peptides (R_8-GRGD). RGD is the ligand of integrin$\alpha V\beta_3$ receptor, which can

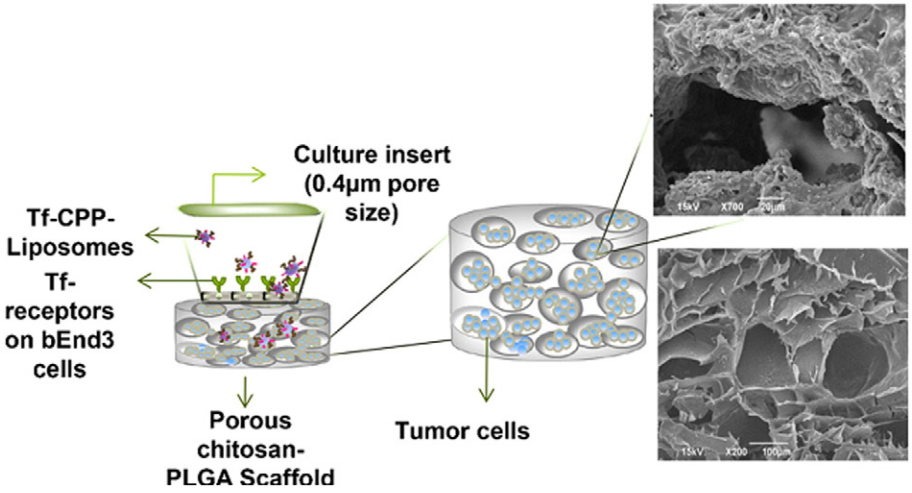

Fig. 3. Pictorial representation of the transport of dual-functionalized (Tf-CPP) liposomes across the endothelial cell barrier. Reprinted with permission from ref. [77].

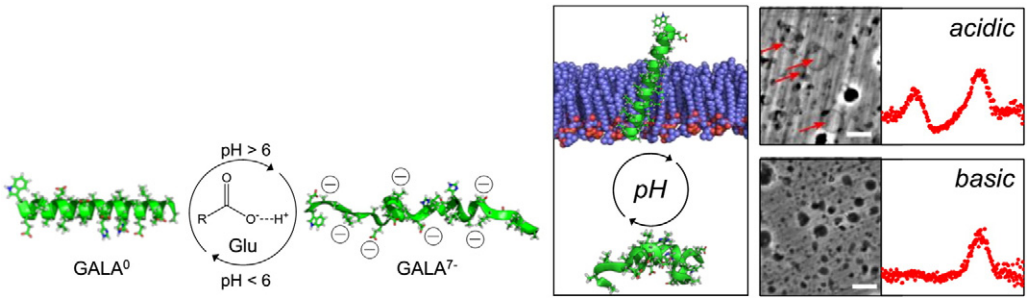

Fig. 4. Reversible folding and unfolding of GALA peptides are triggered by pH for endosomal escape. Reprinted with permission from ref. [80].

target glioma and blood vessels. R_8 is also capable of increasing transportation across the BBB when these R_8-GRGD-mediated liposomes are applied to anti-glioma drug delivery *in vivo* [76].

3.1.2. Synergizing with CPPs for the target drug delivery

The ligand or antibody and CPPs can also be properly dually modified on the nanostructure surface to allow synergistic effect targeting tumor delivery. Three kinds of CPPs were applied to form complexes with the transferrin-liposomes coating the anticancer drug adriamycin. As a result, the transferrin bound to Tf-receptor, significantly increased the targeting capability of fusion molecules, and CPPs improved internalization at tumor cell (Fig. 3) [77]. An immune liposome was also designed, whose surface-modified McAb 2C5 enabled fusion molecules to be localized at tumor cellular surface-bound nucleosomes, with the synergistic TAT executing penetration ability [78].

3.2. Controlled and sustained release based on CPPs

The controlled and sustained release of preparations can be developed depending on the CPPs combining with the physiological environment of tumorigenesis. Some nanoparticle-delivery systems controlled by the tumor microenvironment have been designed, which display a response mechanism that enables hidden CPPs to expose themselves for activation, resulting in the coated drug in complex with the CPP to exert their effect only under the stimulus of low pH, hyperthermy, or interaction with specific enzymes in tumor tissue.

3.2.1. Modulating the activity of CPPs with pH-responsive

At pathologic conditions, tumor has a lowered pH microenvironment in contrast with normal tissue. The approach to harness the cellular uptake of CPPs for tumor internalization by activating CPPs function in response to this low tumor pH can be designed. Two main ways can be applied to modulate the activity of CPPs with pH-responsive. One is using some materials to temporarily shield the CPP ability, and these materials, such as the enteric polymer [79] and the hydrazone bond [78], are pH-sensitive which can be the pH-switch to reveal the CPPs. The other is designing some CPP-self pH-responsive peptides [80,81] or CPPs modified with leucine/histidine sequence [82].

Following these ways, Erez et al. engineered the pH-sensitive PEGylated long-circulating liposomes. The pH-sensitive hydrazone bond (PEG_{2K}-Hz-PE) and TAT were modified on the surface of liposomes. At normal pH, TAT was hidden by the long PEG chains. Upon the exposure to the acidulating environment of solid tumors, the hydrazone bond could be degraded and consequently TAT moieties were exposed enabling permeation of Tat carrying adriamycin into tumor cells [78].

Aside from using the pH-sensitive stealth coating to hide the CPP ability, GALA, a designed synthetic pH-responsive amphipathic peptide, is also applied in drug delivery. GALA is an EALA-repetition peptide, which assumes a random coil or α-helical structure depending on the pH of the environment (Fig. 4). At pH<6, GALA adopts α-helical secondary structure, which can insert into membranes and cause membrane leakage. At pH>6, its glutamic acid side chains deprotonate and become negatively charged, and consequently destabilize the helix structure, which is membrane inactive. This ability of pH-triggered cell-penetration makes GALA-based delivery an efficient strategy for endosomal escape and targeting tumor [80].

3.2.2. Activating CPPs' function under thermal stimulus

The method of controlling CPPs' activity relying on the thermal features of tumor is also used to enhance cellular uptake of drug in the tumor. For example, CPPs in complex with doxorubicin (CPP-DOX) was to coat into thermosensitive liposomes (TSLs), which raised the circulation stability of CPP-DOX in the blood, and enabled the CPP to recover its permeability with DOXduring liquid–solid phase transition of the TSL followed by stimulation of tumor at high temperatures (40 °C to 42 °C) [83]. In addition, a nanopeptifier that amplified cellular uptake by modulating the activity of CPPs with thermally toggled self-assembly of a genetically encoded polypeptide nanoparticle was constructed [84,85]. The nanopeptifier could tune the cellular uptake and activity of anticancer therapeutics by an extrinsic thermal trigger. When appended with a proapoptotic peptide, the nanopeptifier created

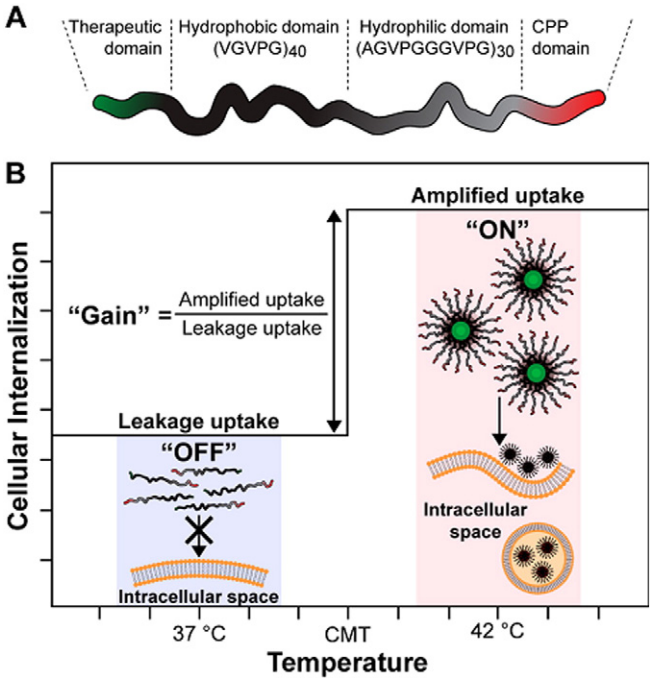

Fig. 5. Design and function of the nanopeptifier. (A) Nanopeptifier was a ternary fusion of a therapeutic payload, an ELP_{BC} composed of a hydrophobic and hydrophilic ELP domain, and a CPP. (B) At "off" state, 37 °C, nanopeptifiers were soluble unimers, displaying a single CPP on their hydrophilic terminus. At "on" state, 42 °C, nanopeptifiers self-assembled into spherical micelles, displaying a high density of CPPs on the micelle corona. Reprinted with permission from ref. [85].

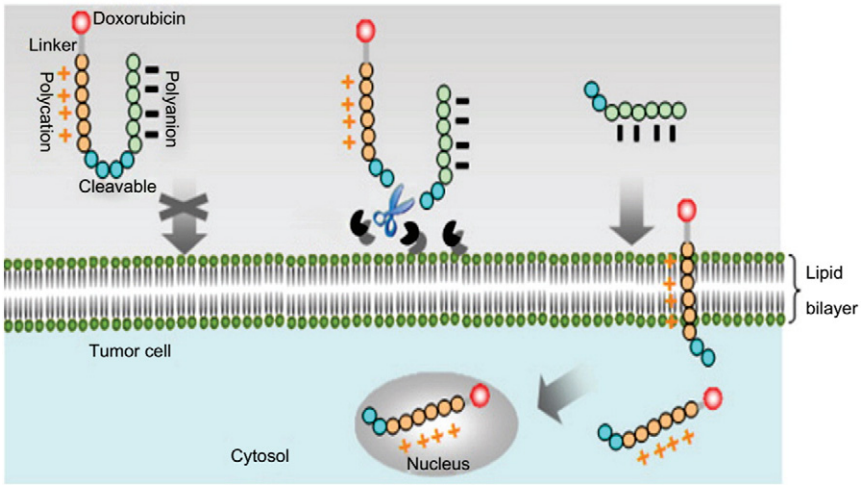

Fig. 6. Schematic diagram of ACPP-DOX conjugate for antitumor drug delivery. Reprinted with permission from ref. [86].

a cytotoxic switch, inducing apoptosis only in its self-assembled state (Fig. 5) [85].

3.2.3. Tune the activity of CPPs by enzyme trigger

An activatable cell penetrating peptide (ACPP) that uses a special control mechanism based on selective and local unleashing of CPP has been extensively applied to tumor therapy and molecular imaging probes. The mechanism of controlled release is mainly through appropriate design of linker, making ACPPs to direct toward particular enzymes, such as matrix metalloproteinases (MMPs) [86], thrombin [87] and legumain [88].

Shi et al. devised a hairpin shaped molecule consisting of a polycationic CPP and an inhibitory polyanion connected through a MMP-specific substrates. When intact, the polyanion neutralized the polycation and largely masked the ability of CPP. Cleavage of the linker enabled dissociation of the inhibitory polyanion from CPP, releasing the CPP and associated cargo to adhere to and then penetrate into tumor cells (Fig. 6). This ACPP-DOX system was temporarily inactive in humoral circulation, but displayed its activity only in tumor tissue overexpressing the MMP-2/9 enzyme [86].

Aside from the release of CPPs from polyanion inhibitors, the CPPs' function can also be activated by the removal of shield coating to reveal CPPs. Ze et al. designed a drug carrier through the attachment of substrate of legumain, alanine–alanine-asparagine (AAN), to TAT. The addition of the AAN moiety to the fourth lysine in the TAT created a branched peptide moiety, which led to a decrease in the transmembrane penetration capacity of TAT by 72.5%. Legumain efficiently cleaved the AAN from TAT and thereby recovered the ability of TAT. Doxorubicin carried by the AAN-TAT-liposome led to an increase in the tumoricidal effect of doxorubicin and a reduction in its systemic adverse effects [88].

3.2.4. Activating CPPs' function in response to extrinsic light trigger

Above experiments controlled the ability of CPP to be activated by the *in vivo* tumor microenvironment. There was also a method involving near-infrared or ultraviolet light, which illuminated tumors to stimulate separation of photosensitive groups (PG) from nanoparticle pcCPP/NGR-LP, thus controlling the release of therapeutic agents at the tumor site (Fig. 7) [89,90].

These mechanisms of "microenvironment-stimulus-response" control the CPP permeability *in vivo* and *ex vivo* and consequently control the drug release. They can significantly enhance the selective penetration of CPPs-drug to tumor or tumor microenvironment and decrease the damage of drug to normal tissues. In addition, they can also improve the sustainable-cure time window of drugs in systemic blood circulation and decrease the danger of CPP degradation by proteases. So CPPs associated with these stimulus-responsive mechanisms can develop the multifunctional drug delivery platform with controlled and sustained release.

In summary, the synergistic or combined effects of CPPs with other delivery techniques can be developed into a MDDS to promote the curative effects of macromolecular drugs (Fig. 8).

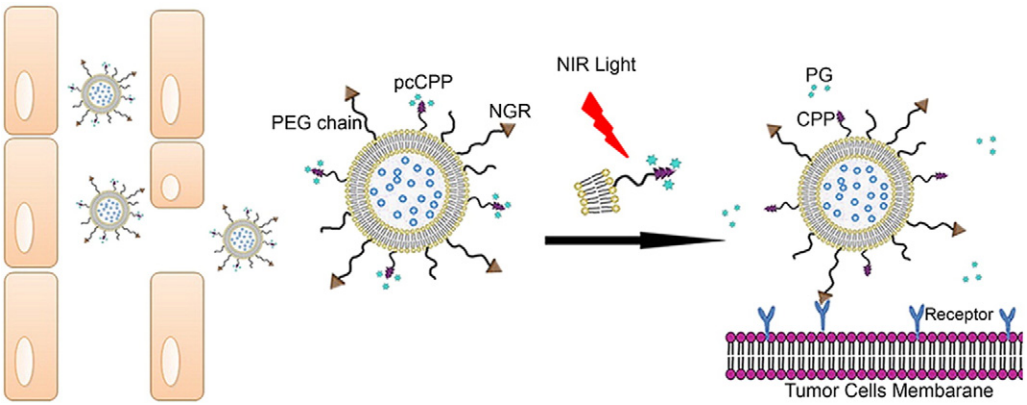

Fig. 7. Schematic illustration of pcCPP/NRG-LP. Reprinted with permission from ref. [89].

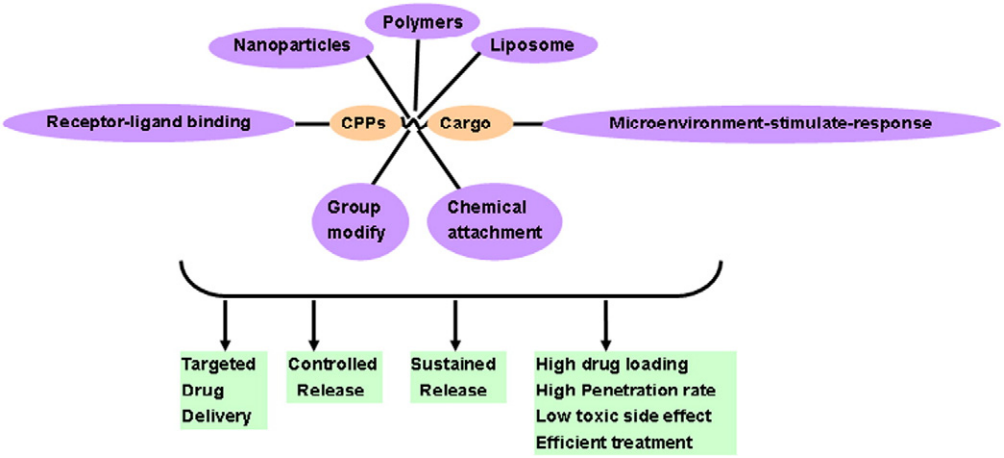

Fig. 8. CPPs incorporating with other vectors develop new multifunctional drug delivery system.

4. The penetrating effects of CPPs on typical biomembrane structure

Studies indicated that various CPPs had different internalizing capacities through the plasma membrane, with some capable of penetrating biomembranes, such as the BBB, gastrointestinal tract, and skin. This activity enables the transport of macromolecules into different tissues and organs, including the brain parenchyma, gastrointestinal circulation, and hypodermis to display their biological function (Fig. 9).

4.1. Penetrating effect on BBB

The BBB selectively allows solutes to cross the barrier and blocks harmful materials entering cerebral circulation in order to maintain the normal physiological state of the central nervous system (CNS) [91]. However, the BBB also forms an impassable barrier against macromolecular drugs designed for CNS-related diseases, restricting their clinical application. Research and development of suitable methods to enable delivery of drugs across the BBB are needed [92,93].

The primary defects of pharmacological and physiological methods for drug delivery include the following: 1) low concentration of drug permeate into brain parenchyma; 2) destruction of BBB enables entry of other toxicants into brain tissues, followed by increased infection probability and tumor migration; 3) increasing liposolubility of the drug may decrease its solubility; and 4) CNS damage cannot be repaired [94].

The application of CPPs for crossing the BBB is a hopeful way to overcome some of these defects. Differences in the abilities of Tat, penetratin, and mastoparan transport of anticancer drugs across the BBB were observed [77]. Tat loaded with siRNA against Raf-1 protein kinase and the anticancer drug camptothecin (CPT) was delivered through the nasal cavity to kill intracerebral malignant glioma cells and extend the mouse life span (Fig. 10) [62]. Additionally, penetratin was able to help RGD permeate SH-SY5Y cells *in vitro*, and penetratin-RGD successfully crossed the BBB, reaching to the ischemic hemicerebrum following a peritoneal injection into local ischemic-reperfusion mice [95]. D-penetratin/L-penetratin was also able to deliver insulin into the cerebral cortex, cerebellum, and brain stem by nasal-cavity administration. The time-effect curves of pharmacokinetics between intravenous and pernasal delivery were compared and analyzed, with the result indicating that pernasal administration of CPPs significantly decreased the danger caused by drug exposure of whole body [96].

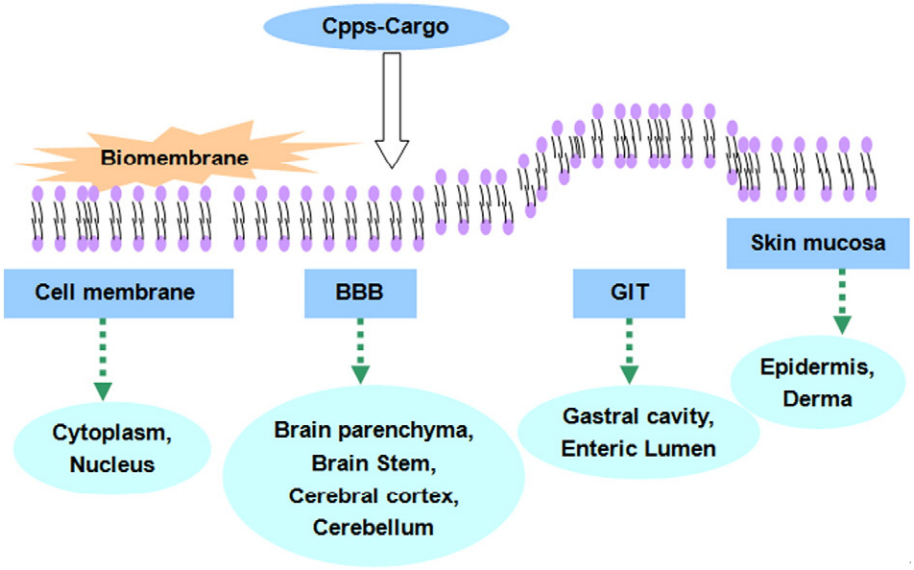

Fig. 9. CPPs-cargo translocate across different biomembranes.

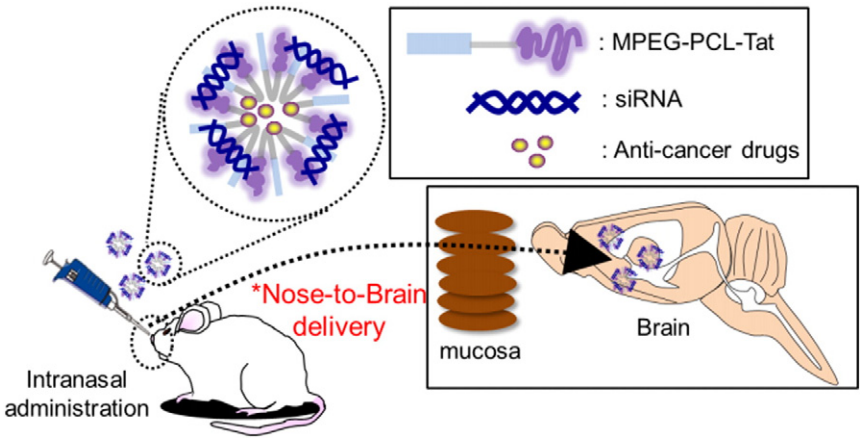

Fig. 10. Intranasal drug/siRNA co-delivery to the brain with TAT-modified nanomicelles. Reprinted with permission from ref. [62].

4.2. Penetrating effect on gastrointestinal mucosa

The mucosa of gastrointestinal tract is a bottleneck for the absorption of biomacromolecules following oral administration. Polypeptides and proteins are often delivered intravenously due to their easier denaturation in the endogastric acidic environment and degradation by intestinal enzymes. Therefore, new effective methods to raise the oral bioavailability of polypeptides and protein drugs are urgently required [97].

CPPs can promote the absorption rate of orally administered protein drugs *via* intestinal epidermal mucosa, and also be a safer drug-delivery method. Mice orally administered penetratin and lipopolysaccharide for seven days, consequently which exhibited the enhanced oral absorption and the safety absorption by analyzing glutamate pyruvate transaminase, glutamate oxalacetate transaminase, and the degree of liver injury [98]. Additionally, it was proved that Tat and penetratin promoted the permeation and intake of insulin into the intestinal epidermal mucosa by using a Caco-2 monolayer cell model *in vitro* [99]. By means of further decreasing the probability of proteolysis and hepatorenal clearance rates, CPPs could also be utilized to enhance the penetrating efficiency and absorption rate of biologics into gastrointestinal mucosa *in vivo*, optimizing the absorption, distribution, metabolism, and excretion process of protein drugs [100].

4.3. Penetrating effect on skin

Skin is a natural barrier that protects the body against external injury. However, macromolecular penetration of the skin is a significant challenge when it is necessary to treat skin diseases, including psoriasis, allergic dermatitis, and skin cancer [101]. Some CPPs, such as meganin, Tat, TD-1, and penetratin, were found to mediate siRNA and protein-like biologics crossing the corneum to enter subcutaneous tissue, thus increasing the absorption efficiency of target cells [102,103]. Among these CPPs, local administration of TD-1 increased the systematic uptake quantity of drugs [53], and Tat was able to effectively deliver gene drugs into epidermal stem cells [59]. In contrast to penetratin and LMVP, melittin is a cationic antibacterial protein, capable of penetrating the abdominal skin corneum to reach the dermis, and also exhibits curative effects on non-melanoma cancer and skin infection [104]. The SPACE peptide was screened by phage-display technology, and displayed the ability to carry protein and to penetrate keratinocytes, fibrocytes, and endothelial cells *in vivo*. It was also able to transport siRNA across the skin corneum to downregulate the expression of target protein [105]. These studies indicated that some CPPs possess the ability to cross the skin barrier, and have a great potential for development of new skin-delivery methods for biological medicine.

In brief, with the ability of biomembranes penetration, CPPs can develop various administration routes for protein and nucleic-acid pharmacon, enhance and improve their clinical application (Table 2).

5. Conclusions

CPPs are capable of transporting macromolecules across biomembranes, enabling their localization to the cytoplasm, cell nucleus, and various tissues for execution of their different functions as follows: crossing the BBB in order to attack CNS diseases; penetrating intestinal mucosa to raise drug absorption rates and oral bioavailability; and permeating skin mucosa to develop percutaneous delivery dosages of protein and nucleic-acid drugs for clinical use. The penetration capacity of CPPs can also be used for studies of the intracellular mechanisms and functional effects of biomolecules.

Currently, the main limitations in CPPs application are easy to be cleaved and degraded by plasma proteases, as well as their lack of specificity, which cause CPPs to lose membrane-permeation ability. Through the addition or substitution of key amino acids, or functional group connection or modification, CPPs can be further remodeled to increase function, and incorporated into other delivery techniques in order to exert synergistic or combined influence. Modified CPPs can increase drug permeating efficiency, facilitate efficient endosomal escape, strengthen the stability of fusion molecules in blood, improve tumor-

Table 2
Different administration routes of CPPs-cargo.

Composition	Administration routes	Efficacy	Function	Ref
TAT-siRNA-CPT	Intranasal administration	Crossing BBB	Kill intracerebral malignant glioma	[62]
Penetratin-RGD	Intraperitoneal injection	Crossing BBB	Reach the ischemic hemicerebrum	[95]
L-penetratin-Insulin	Intranasal administration	Crossing BBB	Decrease the danger caused by drug exposure of whole body	[96]
TAT-Insulin	Orally administration	Crossing gastrointestinal mucosa	Raise the oral bioavailability of insulin	[99]
TAT-LPS	Orally administration	Crossing gastrointestinal mucosa	Prove the safety of CPPs as oral absorption enhancer	[98]
SPACE-siRNA	Percutaneous administration	Crossing skin mucosa	Increase the absorption efficiency of drug to subcutaneous tissue	[105]

CPT, camptothecin; LPS, lipopolysaccharide; SPACE, skin penetrating and cell entering.

tissue targeting, and endow cargo-controlled release with stimulus-responsive mechanisms specific for tumor microenvironments.

With the advance of research and development, more optimized CPPs will be discovered and constructed. Moreover, the integration of various advantages with CPPs will create higher effective and convenient multifunctional drug delivery system, which is the crucial to their further clinical application and promotes new drug research.

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