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REVIEW ARTICLE

A Review on Cell Penetrating Peptides: Golden Chariots for Anti-Cancers Agents Delivery in Cancer Therapy

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KEYWORDS

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ABSTRACT: Cancer has been one of the most prevalent causes of death around the world and despite the development of new therapeutic methods, traditional treatments including chemo and radiotherapy are still the pillars of cancer treatment. However, serious side effects and invasiveness limit the application of traditional cancer methods. To reduce the debilitating side effects of chemotherapy agents, targeted delivery of drugs to malignant cells has been the focus of much research in several past decades. Such efforts however have to overcome serious obstacles. Targeted delivery strategies have been applied to a wide range of cargos including nucleic acid base therapeutic molecules, peptides, and proteins as well as small molecule drugs. Recently, Cell Penetrating Peptides (CPPs) have been indicated as ideal transporters due to their desirable transitional features. CPPs are small synthetic or organic vehicles with the ability to form covalent or non-covalent bonds with a wide variety of substances. The capability of CPPs in crossing cell membranes, has received well-deserved attention in designing novel diagnoses and therapeutic strategies. In this review article, we compared structurally similar carriers designed by different research groups and discussed their strengths and limitations. The comparison was mainly based on the sequence properties of CPPs. We further discussed CPPs according to their cargo and reviewed their application in "*in vivo*" research. Research shows that despite the significant progress, drug delivery by CPPs has to address several issues, and such challenges have paved the way for further studies in the CPP field.

INTRODUCTION

Cell Penetrating Peptides (CPPs) are small peptides with positive charges (fewer than 30 amino acids) [1, 2] , which can cross through the negative-charged phospholipid membrane and transport cargo. Also, their penetration depends on their sequence [3]. They are also known as protein transduction domains (PTDs) [4]. Chimeric peptides are a group of CPPs, which are expected to be more efficient carriers because they are engineered to pass cell obstacles. These easily-constructed and safe peptides consist of several connected fragments chosen for a special purpose. One fragment has membrane-penetrating specifications, the other fragment has cargo interaction properties and also there is one more fragment for targeting the goal zone which is cancer cells.

For years different patients have been treated with various anti-cancer methods like chemotherapy drugs. These common cancer therapy agents cause side effects [5]. Since chemotherapy drugs can target normal cells too, it is so important to deliver drugs to cancer cells specifically. To reduce the drug distribution and hence the side effects of chemotherapy agents, targeted delivery

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of chemotherapy agents to malignant cells has been the subject of focus for decades. Several approaches including targeting carrier peptides toward tumor-specific antigens or thermal / pH-sensitive carriers have been applied so far [6-8]. Targeted drug carriers, which can find cancer cells, increase the accumulation of drugs in the cancer zone and decrease chemotherapy drugs' side effects on healthy cells [9, 10]. Having a specific fragment for cargo attachment, chimeric peptides have been used to transport various anti-cancers such as plasmid, siRNA (small interfering RNA), miRNA (microRNA), proteins, chemotherapy drugs, and even anti-cancer loaded nanoparticles (NPs) [11] (Figure 1).

Here we reviewed the advances in the application of CPPs as delivery systems specifically in cancer treatment. We first categorized research articles based on the biochemical composition of the cargo and then we reviewed the *in vivo* application of CPPs. Based on our findings, CPPs have opened a wide horizon in targeted drug delivery to specific sites especially malignant cells. However, much research is still needed to address issues including cell type, specificity poor stability in the bloodstream as well as low bioavailability in target tissues before CPPs become a made-strategy in the drug delivery world.

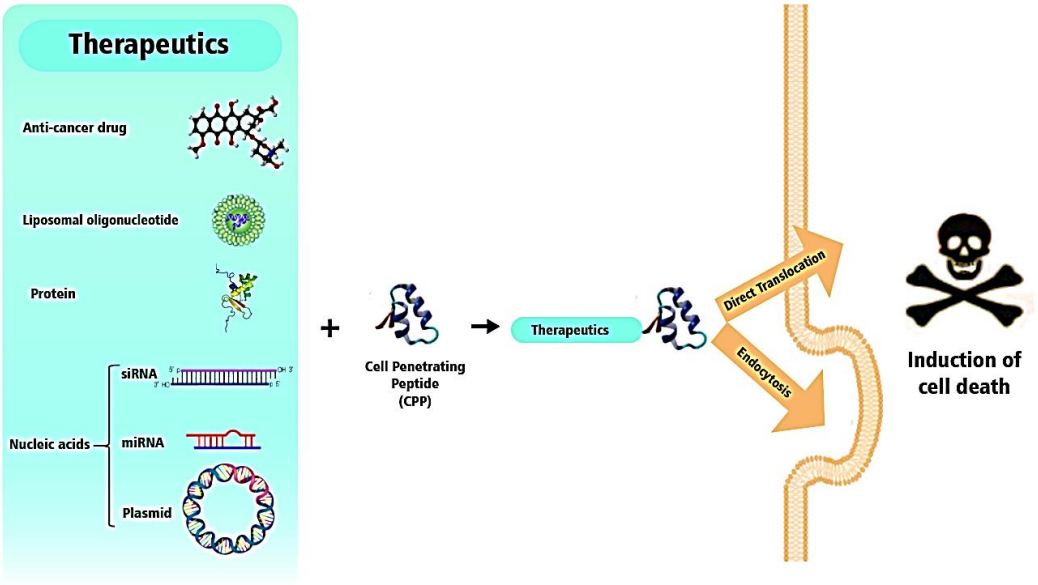

Figure 1. A schematic figure of CPP, which carries possible cargo. These cargos can be siRNA, miRNA, plasmid, proteins, and chemotherapy drugs.

Recent advancements in the application of CPP in cancer therapy

Traditional cancer treatments like surgery and chemotherapy are still the most frequently used methods. These invasive cancer therapies seem not to be effective due to their serious side effects. Designing novel delivery systems like CPPs, has overcome many of the classic treatment difficulties. For example, minimizing side effects by selective cancer cells delivery, which also increases the accumulation of the drug in the target and decreases the total dose compared to traditional strategies. To target cancer cells selectively, they need to be correctly recognized. Expression patterns of surface markers are different in normal and cancer cells, some of these markers are overexpressed in cancer cells which are ideal targets for CPPs. As an example of the application of CPPs in cancer treatment, a group of researchers studied skin cancer and designed a CPP (NRPDSAQFWLHH) that carried virus-like NPs, derived from hepatitis B (VLNP), targeting epidermal growth factor receptor (EGFR) markers and entering A431. A high rate of CPP complex internalization towards A431 cells has been observed [12]. EGFR is a marker overexpressed in cancers like squamous cell

carcinoma, non-small cell lung cancer, and gastric, esophageal, colorectal, prostate, renal, bladder, ovarian, and pancreatic cancers [12]. In ovarian cells, as said above, except for EGRF, there is another marker called T7 peptide (HAIYPRH). This transferrin receptor targeting ligand, caused the uptake of T7-decorated Nps encapsulated seliciclib, by MDA-MB-231 cells (overexpress transferrin receptor) more than SKOV-3 cells and U87-MG cells, which expressed lower levels of transferrin [13] .

Liposomes are one of the novel drug deliveries in cancer treatments that have attracted researchers' attention these days. For example, a dual-functional liposome was designed. This paclitaxel (PTX)-loaded liposome was decorated with RGD (arginine-glycine-aspartic acid) and bound to integrin and was a ligand to the human transferrin receptor (TFR). PTX-loaded RGD/TF-LP (transferrin liposome) entered A2780 ovarian carcinoma cells via macropinocytosis and delivered PTX and exhibited anti-glioma effects successfully [14]. In a comparative study, several chimeric peptides were designed and used to make their liposome selective. These peptides consisted of R8 (8 arginines), R8-RGD, R8-EGR (early growth response protein), and R8-dGR (reverse RGD). Results showed that the complex consisting of R_8 and a conjugated RGD reverse sequence, DGR, could successfully bind to both integrin $\alpha_V\beta_3$ and neuropilin-1 receptors and transfer PTX efficiently. These two types of receptors are overexpressed in C_6 brain cancer cells and mice model [15]. In another study, a novel complex system consisting of synthetic peptides and a siRNA cargo was designed. The complex included R9 (9 arginines) and myr (hydrophobic myristoylated amino-terminus) which could connect to LyP-1 or iRDG. Among these two motifs, iRDG was an identifying ligand for integrins. LyP-1 was a ligand for p32/gC1Qr. LyP-1 and iRDG could similarly recognize neuropilin 1. These peptide / siRNA complexes were delivered to MDA-MB-231 and could suppress these breast cancer cells successfully [16]. In some liposome-CPP complexes, internal or external triggers cause CPP separation in the target site. Also, a nanobubble was synthesized from Polyethylene glycol (PEG) which was decorated with NGR (Asparagine-Glycine-Arginine). These nanobubbles were carrying a CPP (CKRRMKWKK) conjugated to doxorubicin (DOX). This complex reached fibrosarcoma cells (HT-1080) with the help of NGR. Then an external trigger (uv irradiation) caused the release of CPP–DOX complex from the nanobubble and entered the cell via vascular antigen aminopeptidase N (CD13). Then DOX caused cancer cell death [17]. This external trigger can be heat too. It was synthesized a penetrating-derived CPP (CKRRMKWKK) targeting CD13 receptors of fibrosarcoma cells (HT-1080). In a similar study, scientists used heat as an external trigger and proved that DOX released from this CPP at 42 degrees and stopped HT-1080 proliferation successfully [18] .

Nucleic acids delivery to cancer cells by CPPs

Some of the cancer therapeutics such as siRNA (small interfering RNA), miRNA (microRNA), plasmid DNA, and ASO (antisense oligonucleotide) have nucleic acids, which can be carried by CPPs. Due to their positive charges, cationic CPPs can interact with anionic oligonucleotides electrostatically while hydrophobic interaction between oligonucleotides and amphipathic CPPs consisted of hydrophilic and hydrophobic domains [1]. Some CPP-based nucleic acid delivery systems are summarized in Table 1.

siRNA is a small (20-27 base pairs) double-strand, non-coding RNA, which in the cell incorporates with some proteins to form RISC (RNA-induced silencing complex) and changes to single-strand RNA, which can find its complementary mRNA and bind to it then induces mRNA cleavage and degradation, in other words, it can silence gene expression [19]. As it was indicated previously, the expression of some proteins increases in cancer cells. Transporting a specific siRNA to these cells silences the related protein expression, which can even be effective in the apoptosis pathway and cancer cell death. It was constructed a CPP, named 599, which consisted of R9 and fusogenic peptide motif derived from influenza virus (INF7) (GLFEAIEGFIENGWEGMIDGWYGGGGRRRRRRRRRK). 599 was used to deliver CIP2A (Cancerous inhibitor of protein phosphatase 2A) siRNA to CAL 27 and SCC-25 (Squamous cell Carcinomas) oral cancer cell lines. CPP-siRNA was internalized by cells via endocytosis. The target, CIP2A, is an oncoprotein

overexpressed in oral cancer cells and its suppression causes tumor growth inhibition [20]. 599 were used in a similar study to deliver CIP2A-specific siRNA to a xenograft oral cancer mouse model. The results indicated that 599 was able to protect siRNA from ribonucleases and successfully support the endosomal escape of the siRNA which resulted in successful silencing of CIP2A and tumor growth inhibition [21]. As mentioned previously, EGFR is among cell surface receptors with high expression levels in several cancers including oral cancer. Results from a related study showed that when 599 was fused to an EGFR targeting peptide (GE11R9), it was successful in delivering CIP2A-specific siRNA to CAL 27 and SCC-15-cells. The carrier delivered siCIP2A to cells with EGFR markers via endocytosis and eventually an impressive gene silencing was observed [22].

The use of CPPs as delivery vehicles for siRNA has been broadened by combining CPP-siRNA complexes with micromodels. For example, R8/siBRAF-coated microneedles were used to suppress BRAF in A375, a melanoma cell line. Results showed effective suppression of the BRAF gene and induction of apoptosis. Cell proliferation was successfully inhibited as well [23]. The interaction of cargo-carrier in R8/siBRAF coated MNs was non-covalent. Non-covalent interaction was used in another study as well. It was showed that breast cancer stem cells (CSC) which express high levels of CD44 show high activity for Feline sarcoma-related tyrosine kinase (FER). Therefore, it was synthesized a complex consisting of FER siRNA linked to low molecular weight Protamine (LMWP). LMWP/siFER was able to knock down the FER mRNA and inhibit cell proliferation in human breast cancer MDA-MB-231 cells CD44 High cells [24]. LMWP has also been used as a carrier for siVEGF (Vascular endothelial growth factor) delivery to cancerous and non-cancerous cell lines. LMWP/siVEGF did not exhibit cancer cell line specificity. To address this issue researchers designed a CPP called BR2 (RAGLPFQVGRLLRRLLR). SiVEGF/BR2 was able to down-regulation of intra-cellular VEGF levels and tumor growth suppression in cancerous cell lines successfully [25]. In another successful study, NP1, a novel CPP was designed that consisted of stearic acid, 16 histidine residues, and R8. This CPP successfully transferred Bcl2-siRNA to 3D spheroids of colon cancer cells (HCT 116). Bcl2-mRNA knockdown, apoptosis induction, and angiogenesis inhibition were observed [26]. A newly designed CPP, S413-PV, which consisted of Acetyl-ALWKTLLKKVLKAPKKKRKVC-NH2) linked to an acyl group (C12), was able to deliver siGFP to U87 cell line stably expressing green fluorescent protein (GFP). Results showed a successful knockdown of GFP expression [27]. A reciprocal translocation in the Bcr-Abl gene causes constitutive tyrosine kinase activity in chronic myeloid leukemia. Novel-designed CPPs pepM (KLFMALVAFLRFLTIPPTAGILKRWGTI) and pepR (LKRWGTIKKSKAINVLRGFRKEIGRMLNILNRRRR) in combination with several designer siRNAs demonstrated an efficient siRNA delivery and knockdown of Bcr-Abl gene to BV173 cells. As a result, proliferation was inhibited successfully [28]. Antisense oligonucleotides (ASOs) are synthetic single strands DNA oligomers that couple with complementary mRNA and down-regulate gene expression or bind to microRNAs and inhibit them [29]. Researchers also worked on miR-21 role overexpressing in glioblastoma tumor cells. Matrix metalloproteinase (MMP) regulators are affected by miR-21 and promote glioma invasion [30]. Inhibiting miR-21 by anti-miRNA, carried by an arginine-rich CPP (the complex called: anti-miR-21/R8), decreased glioblastoma migration and increased cancer cell death [31]. In another study transferring miR-21 antisense oligodeoxynucleotide to C6 and A172 glioblastoma cells by amphiphilic R3V6 peptide-induced apoptosis and programmed cell death 4 (PDCD4). Surprisingly the rate of transfection by R3V6 was more than transfection by polyethyleneimine or lipofectamine [32]. *In vivo* study on the C6 glioblastoma xenograft mice model confirmed the obtained results of the mentioned *in vitro* study [33]. A lipid nanocomplex (Lp-PPRP) composed of 8 arginines modified with palmitic acid, a polyethyleneimine, and an antisense oligonucleotide (LOR-2501) was used to inhibit the mRNA of R1. R1 is a component of ribonucleotide reductase and an important target of anti-tumor drugs. An *in vitro* study on Hela and A549 cancer cells showed the transfection efficiency of this nano-complex was more than polyethyleneimine as a carrier. LOR-2501

down-regulates the expression of R1 protein and plays a role in cancer therapy [34].

MicroRNAs are single-strand, non-coding, gene regulator RNAs, which can regulate gene expression via coupling with complementary mRNA [35]. miR-126 is a tumor suppressor downregulated in many cancer cells. A short penetration accelerating sequence (FFLIPKG) or PAS, was covalently attached to 15 amino acids in the N terminal of mitochondria-bound hexokinase II (MIASHLLAYFFTELN) or pHK. This novel CPP was used to transfer miR-126 to MCF7 breast cancer cells and caused cancer cell death. miR-126 has also been downregulated in pancreatic cancers [36]. miR9 is categorized as tumor suppressor microRNAs. Decreased levels of miR9 have been reported in pancreatic cancers. Downregulated levels of miR9 result in elF5A2 upregulation. It was designed a plectin 1 targeting peptide for the targeted delivery of miR9 to a mouse xenograft model of pancreatic cancer. Apoptosis was successfully induced. Additionally, DOX sensitivity was increased in pancreatic ductal adenocarcinoma (PDAC) [37]. The expression level of USP9X (ubiquitin-specific peptidase 9, X-linked) is in part controlled by miR-212. USP9X has an important role in DOX resistance and regulation of epithelial-mesenchymal transition (EMT), apoptosis, and autophagy of pancreatic ductal adenocarcinoma cells. miR-212 delivery via a chimeric peptide composed of arginine-rich fragments for miR-212 condensation, and plectin-1 for targeting pancreatic ductal adenocarcinoma cells, decreased USP9X-expression and increased DOX-induced apoptosis and autophagy of pancreatic ductal adenocarcinoma cells [38].

CPPs have been used in plasmid DNA (pDNA) delivery as well. It was designed a CPP for pDNA delivery to HT1080 and 4T1 mouse tumor models. This plasmid was designed to express short hairpin RNA (shRNA) against vascular endothelial growth factor (VEGF). PF144 (Stearyl) AGYLLGKLLOOLAAAALOOLLXPLGLAGPEG600NH2) was designed to be activated by MMP2 and 9. PF144 was able to deliver anti-VEGF shRNA coding plasmid to cancer cells. VEGF was successfully knocked down which in turn led to inhibition of tumor [39].

Table 1. Various CPP-based nucleic acid delivery.

CPP	Cargo	Model	Function	Reference
599 Peptide (R9 and fusogenic peptide motif derived from influenza virus (INF7))	CIP2A siRNA	*In vitro* (oral cancer cell lines)	Enhancement of CIP2A gene silencing / Decrease of oral cancer cell invasiveness	[20]
599 Peptide	CIP2A siRNA	*In vitro* (oral cancer cell lines) /In vivo (Xenograft oral cancer mouse)	Enhancement of CIP2A gene silencing / Tumor growth inhibition	[21]
Dual peptide (599 & GE11R9)	CIP2A siRNA	*In vitro* (oral cancer cell lines)/ In vivo (Xenograft oral cancer mouse model)	Enhancement of CIP2A gene silencing / Inhibition of tumor growth	[22]
R8	BRAF siRNA	*In vitro* (melanoma cell line)/ In vivo (Murine Melanoma model)	suppression of BRAF gene / Inhibition of proliferation / Induction of apoptosis	[23]
Low molecular weight protamine	FER siRNA	Human breast cancer cell line	Inhibition of cancer migration and colony-forming ability	[24]
BR2	VEGF siRNA	Human cervical/colon cancer cells, mouse fibroblast cells, and human keratinocyte cells	Downregulation of intra-cellular VEGF levels in cancer cells / Improvement of antitumor efficacy without toxicity	[25]
NP1	Bcl2 siRNA	Colon cancer cells	Apoptosis induction and angiogenesis inhibition	[26]
S413-PV	GFP siRNA	Stably GFP expressing cell line	Knockdown of GFP expression	[27]
Pep M and pep R	Bcr-Abl siRNA	Chronic myeloid leukemia	Downregulation effects on proliferative CML-related signaling pathways	[28]
R8	Anti-miR-21	Glioblastoma cell line	Decrease of cell migration and increase in cancer cell	[31]

			death	
R3V6	Anti-miR-21	Glioblastoma cell line	Apoptosis induction	[32]
R3V6	Anti-miR-21	C6 glioblastoma xenograft animal model	Inhibition of miR-21 / promotion of expression of the programmed cell death 4 (PDCD4) gene/induction of apoptosis	[33]
PAS-pHK	miR-126	Breast cancer cells	Apoptosis induction	[36]
PL-1 and SP-94R	miR-9	*In vitro* (Pancreatic ductal adenocarcinoma cells)/In vivo (Patient-Derived Xenografts (PDX) mouse model)	improve the anticancer effect of DOX through downregulating eIF5A2 expression to inhibit autophagy and induce apoptosis	[37]
PL-1 and SP-94R	miR-212	*In vitro* (Pancreatic ductal adenocarcinoma cells/ normal pancreatic cell line/ hepatocellular carcinoma cell line/ lung cancer cell lines)/ In vivo (Patient-Derived Xenografts (PDX) mouse model)	Decrease of USP9X expression/ induction of apoptosis and autophagy of PDAC cells	[38]
PF144	Plasmid (anti-VEGF shRNA)	Mouse tumor models	Inhibition of tumor growth by silencing VEGF expression	[39]

Protein delivery to cancer cells by CPPs

According to previous research, human papillomavirus E6 and E7 oncogenes are expressed in most cervical cancers while the expression of the E2 regulatory gene is disrupted. Therapeutic approaches based on E2 delivery to Hela, C-41, and MS751 cells, successfully inhibited E6 and E7 gene expression, cell growth, and proliferation [40]. Recently a CPP called TAT-CAM was used to deliver E2 protein to HPV-16+ cervical cancer cell line SiHa. TAT-CAM consisted of a calmodulin and the well-known CPP, TAT [41]. E2 protein was engineered to have a calmodulin-binding sequence (CBS). Engineered E2 was attached to TAT-CAM through a non-covalent bond. The complex could successfully deliver E2 and consequently inhibited cellular proliferation and promoted cancer cell death [42].

Drugs and liposomal drugs delivery to cancer cells by CPPs

CPPs have been applied for drug delivery of free or encapsulated chemotherapy agents such as DOX to cancerous cell lines [43]. Elastin-like polypeptides (ELPs) are used as thermo-sensitive vectors for cargo delivery to tumors. A complex consisting of ELP (derived from tropoelastin), a CPP (TAT), a 2-MMP substrate (PLGALG), and a derivative of Dox was designed and constructed. This complex was used for DOX delivery to HT-1080 breast cancer cells. In comparison with ELP-Dox, the mentioned complex showed a 4-fold increase in cell penetration and consequently more death in cancer cells. More importantly, even in encountering DOX-resistant cells, the complex showed better penetration and caused more cell death [44]. In a related attempt for DOX delivery by CPP, a series of linear and cyclic CPPs for drug delivery to leukemia, ovarian adenocarcinoma, colorectal, and breast carcinoma cell lines was designed. Cyclic CPP (W (RW)4) and linear CPP (RW)4 were covalently conjugated with DOX via a linker and used for cancer therapy. These CPPs showed high cellular uptake and could successfully inhibit cancer cells. Both cyclic and linear DOX- conjugated CPPs were able to inhibit cell proliferation more strongly compared to DOX. Meanwhile, cyclic CPP showed stronger effects in comparison to linear CPP [45].

Researchers have been trying to improve the efficacy of drug delivery by liposomal systems for decades. Liposomal drug delivery to cells can be improved by the conjugation of liposomes with CPPs [46]. For example, penetratin and TAT were used for the delivery of DOX to A431 cells. TAT could increase the intracellular

accumulation of DOX over time. Interestingly, in the case of penetratin intracellular accumulation reduced over time [47]. In this regard, modified pH-sensitive liposomes were decorated by cationic CPP (GGRRRRRRRRR-amide). This drug delivery system was used for DOX delivery both *in vivo* and *in vitro*. Due to the acidic environment of tumors, the remarkable accumulation of DOX in mice tumor models confirmed the success of targeting and penetrating liposomal DOX [48].

It has been reported that the conjugation of CPPs to compatible copolymers can improve the pharmacokinetic/dynamic properties of these complexes. For example, It has been reported that N-(2-hydroxypropyl)-methacrylamide (HPMA) can improve both serum half-life and accumulation of DOX in the tumor tissue [49, 50]. In recently published research, a DOX- conjugated HPMA was attached to dNP2 (CKIKKVKKKGRKKIKKVKKKGRK), a human-derived CPP, for drug delivery to the HeLa cell line. This novel drug delivery complex resulted in a high cellular uptake, DNA damage, and apoptosis in HeLa cells [51].

Conjugation of CPP/liposome has also been applied for siRNA delivery to cancer cells. A complex consisting of an ACCP (activatable CPP) and liposome encapsulating siRNA was synthesized by another research team [52]. ACCP included CPP (octaarginine), a pH-sensitive cleavable linker (hydrazone), and a polyanionic domain (glutamic acid and histidine-rich peptide (ehGehGehGehG)). This complex was designed for polo-like kinase 1 (PLK)-siRNA delivery to MCF7 and A549 cancer cells and tumors. The acidic pH of the tumor environment resulted in hydrazine breakdown and siRNA delivery to its target [52]. BR2 (RAGLQFPVGRLLRRLLR), another CPP, is used for enhancing liposomal cantharidin penetration to HepG2, hepatocellular carcinoma cells. In comparison with unmodified liposomes, BR2-modified liposomes showed significant improvement in penetration and tumor growth inhibition [53].

In vivo successes in cancer therapy by anti-cancer-*CPP complexes*

Despite significant progress in conventional cancer therapy, malignant tumors are still one of the three leading causes of death in human societies. One of the challenges that novel therapeutic approaches face, is the lack of universally effective and specific drug delivery systems. In this regard, the application of CPPs for targeted delivery of cargo to cancer cells *in vivo* has been the focus of pilling research.

Overexpression of the mutated tumor suppressor protein p53 in more than 50% of solid tumors has made p53 an attractive target for cancer therapy. In a phase I clinical trial with p28, patients with metastatic solid tumors were subjected to intravenous injections of p28 peptide. P28 is an anionic CPP, which binds to wild-type and mutant p53 protein and prevents the binding of constitutional morphogenic protein 1 (Cop1) to p53. Consequently, Cop1 decreased through auto degradation and p53 protein increased, inducing cell cycle arrest at G2/M, and inhibiting tumor growth [54, 55]. It was shown that TAT, as a carrier of DOX, was successful in the suppression of SKOV-3, an ovarian cancer cell line. To evaluate the efficacy of TAT application *in vivo*, the amines of TAT lysine residues were amidized to succinyl amides. Amidized TAT was then complexed to PEG-Poly(ε-caprolactone) micelles. The addition of PEG-Poly(ε-caprolactone) resulted in the inhibition of nonspecific interactions in blood circulation. In the acidic environment of tumors in the ovarian cancer mouse model, succinyl amides were hydrolyzed and restored TAT function and fast cellular DOX uptake [56]. These results were in complete agreement with results obtained by other researchers. The injected SKOV-3 tumor xenograft mice were injected with liposomal DOX modified by TAT, anti-nucleosome monoclonal antibody 2C5, and a pH-sensitive polymer. The tumor growth was reduced significantly and therapeutic efficacy was enhanced remarkably in drug-sensitive and drug-resistant ovarian cancer models [41]. It was also used ε-caprolactone in the structure of the star shape NPs. The carrier included Methylene- PEG, an MMP triggered peptide (GPLGIAG and R9), tricarballylic acid, and ε-caprolactone. As it was mentioned before, MMPs are overexpressed in the extracellular matrix of most cancers including lung cancer and as a result, the modified star-shaped NP was able to deliver curcumin to lung tumors and inhibited tumor growth [57].

Another MMP-triggered NP was developed for glioma

cancer. Due to the blood-brain and blood-tumor barriers, glioma treatment with anti-cancers has proven difficult. ACP, a chimeric CPP, was able to pass blood-brain barriers. This CPP consisted of R8 and eight glutamic acid residues which were connected with a matrix metalloproteinase-2-sensitive linker (PLGLAG). This dual-modified CPP was used as a vehicle for drug delivery of docetaxel to glioma-bearing mice tumor model and showed a remarkable anti-glioma effect [58]. An *in vivo* study conducted on brain tumor-bearing female KM mice also confirmed the implication of R8 for an enhanced cell delivery of liposomal DOX. It was revealed that liposomal DOX combined with R8 could increase cellular uptake 8.6-fold compared to uncoated liposomes. In this study, an oleic acid conjugated modified R8 was proven more efficient than R8 [59]. Prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA) are over-expressed in prostate cancer cells specifically at the non-responsive stage. In an experiment polo-like kinase 1 (PLK-1) siRNA was loaded in a dual modified liposome. This drug delivery system was modified with polyarginine (polyR) and decorated with PSA- responsive moiety and PSMA ligands. PSA-/ PSA-ligand interaction resulted in cleavage of PSA and liposome internalization through the polyR's penetrating effect. Due to dual targeting, the complex accumulated in prostate tumors, knocked down PLK-1, inhibited tumor growth in mice, and induced cancer cell apoptosis [60].

In another attempt on prostate cancer treatment, cycB1-siRNA was non-covalently attached to MPG-8. MPG-8 is an improved variant of MPG. MPG is an amphipathic peptide that consists of an N-terminal motif derived from the diffusion sequence of HIV gp-41 and a hydrophilic domain derived from SV-40 large antigen. This drug delivery system was able to successfully deliver cyc-B1 siRNA to mice prostate tumor model and silence cyclin B1 which led to the inhibition of tumor growth [61].

Since the cyclic peptide iRGD can target and penetrate tumor cells via interactions with αvβ3/5 integrins and neuropilin-1, a tandem peptide combining a CPP and optimized iRGD to encapsulate KRAS-siRNA was constructed. These tumor-penetrating nanocomplexes (TPNs) were capable of delivering siRNA to PDAC. They further modified TNPs by pegylation for systemic drug delivery. Their results indicated a significant delay in tumor growth. Additionally, the modular design of their delivery vehicle holds promise for adaptation to diverse genetic target candidates in pancreatic cancer [62]. Successful *in vivo* delivery of siRNA by CPP was also obtained by another study. It was designed a carrier by the fusion of a dsRNA binding domain to TAT and constructed a chimeric CPP, which successfully delivered EGFR and Akt-2 siRNA to the glioblastoma mouse tumor model. Their result indicated a decrease in proliferation in brain tumor-bearing mice [63].

TAT has also been used in a recombinant fusion protein for colorectal cancer treatment. TAT-gelonin fusion protein enhanced gelonin activity against colorectal cancer cells. A combination of TAT-gelonin fusion protein and heparin/anti-carcinoembryonic antigen monoclonal antibody can specifically bind to a carcinoembryonic antigen, which is over-expressed in LS147T colorectal cancer cells. Electrostatic interaction between cationic TAT and anionic heparin constructed a toxin protein delivery complex that targeted colorectal tumors. This platform increased the inhibition of tumor growth in xenograft mice significantly [64].

CPPs have been implied in *in vivo* studies concerning breast cancer as well. It was delivered DOX with another newly designed carrier to mice bearing breast cancer. The overall structure of the vehicle was SynB1 (a CPP) conjugated with DOX and ELP. The structure has an acid-sensitive linker. In tumor acidic environment the linker cleavage released DOX. Results showed potent anticancer effects [65]. Antitumor efficacy of PTX in the MCF7 xenograft tumor model was significantly increased when PTX was loaded on a Liposome/CPP complex. The CPP motif was a hydrophobic CPP, PFV (PFVYLI). As a result, PTX accumulated and antitumor effects were remarkably improved [66]. Successful attempts of delivering anti-cancer by CPPs are briefed in Table 2.

Table 2. Several examples of CPPs used as *in vivo* anti-cancer carriers.

CPP	Sequence	Cargo	Tumor type	Reference
CPP	CKRRMKWKK	siRNA	Breast	[67]
SynB1	RGGRLSYSRRRFSTSTGRA	DOX	Breast	[65]
R9	RRRRRRRRR	Curcumin	Lung	[57]
TAT	GRKKRRQRRRPPQ	DOX	Ovarian Breast	[56]
ACP	EEEEEEEE-6-aminohexanoyl-PLGLAG-RRRRRRRR	Docetaxel	Brain	[58]
MPG-8	AFLGWLGAWGTMGWSPKKKRK	siRNA	Prostate	[61]
TAT	GRKKRRQRRRPPQ	Toxin protein	Colon	[64]
Polyarginine	RRRRRRRR…	siRNA	Prostate	[60]
TAT	GRKKRRQRRRPPQ	DOX	Ovarian	[41]
P28	LSTAADMQGVVTDGMASGLDKDYLKPDD	-	Brain	[54]
599	GLFEAIEGFIENGWEGMIDGWYGGGGRRRRRRRRRK	siRNA	Oral cancer	[21, 22]
R8	RRRRRRRR	DOX	Ovarian	[14]
PFV	PFVYLI	Paclitaxel	Breast	[66]

CONCLUSIONS

CPPs present a novel research horizon in the biomedical area. These small peptide sequences are introduced as novel carrier systems, especially for anti-cancer therapeutic agents for the sake of their noninvasive entry into cellular membranes, biosafety and highly efficient, and so on. In general, various CPPs with different sequences of amino acids have been investigated to deliver therapeutic molecules like nucleic acids, proteins, peptides, and drug molecules. Cell type specificity, poor stability in the bloodstream, as well as low bioavailability in target tissues owing to poor tissue penetration, are the important hindrances to CPP-based treatments. Diverse strategies have been employed to circumvent these issues, including conjugation of CPPs with homing peptides, attachment of targeting ligands (e.g. hyaluronic acid, folic acid, RGD peptides), coupling CPPs with macromolecules (e.g. liposomes or biopolymers). It should be noted that the modification of these peptide sequences could in some cases produce immune responses, whereas a bare CPP did not provoke any immune responses. Consequently, further clinical studies are required for the optimization of the employment of CPPs for the efficacious delivery of anticancer molecules.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

Abbreviation

CPP: Cell penetrating peptides
EGFR: epidermal growth factor receptor
VLNP: virus-like nanoparticles
TFR: The human transferrin receptor
PTX: Paclitaxel
RGD: arginine-glycine-aspartic acid
PTX loaded RGD/TF-LP: Paclitaxel-loaded RGD/transferrin liposome
PTX-PEG-Lip: Paclitaxel-Polyethylene glycol-Liposome
PTX-R8-Lip: Paclitaxel-8 arginines –Liposome
PTX-R8-RGD-Lip: Paclitaxel-8 arginines- (arginine-glycine-aspartic acid) –Liposome
PTX-R8-EGR-Lip: Paclitaxel-8 arginines- early growth response protein -liposome
PTX-R8-dGR-Lip: Paclitaxel-8 arginines-reverse RGD-Liposome
Myr: hydrophobic myristoylated amino-terminus
PEG: Polyethylene glycol
NGR: Asparagine-Glycine-Arginine
DOX: doxorubicin
siRNA: small interfering RNA
miRNA: microRNA
ASOs: Antisense oligonucleotides
FER: Feline sarcoma-related tyrosine kinase
RISC: RNA induced silencing complex
R9: 9 arginnines
R8: 8 arginnines
INF7: fusogenic peptide motif derived from influenza

virus
CIP2A: ancerous inhibitor of protein phosphatase 2A
SCC: Squamous cell Carcinomas
CSC: cancer stem cells
FER: Feline sarcoma-related tyrosine kinase
LMWP: low molecular weight Protamine
VEGF: Vascular endothelial growth factor
GFP: green-fluorescent protein
CML: Chronic myeloid leukemia
MMPs: Matrix metalloproteinases
PDCD4: R3V6 peptide induced apoptosis and programmed cell death 4
Lp: Lipid
PAS: penetration accelerating sequence
PDAC: Pancreatic ductal adenocarcinoma
USP9X: ubiquitin specific peptidase 9, X-linked
EMT: epithelial-mesenchymal transition
pDNA: plasmid DNA
shRNA: short hairpin RNA
CBS: calmodulin binding sequence
ELPs: Elastin-like polypeptides
HPMA: N-(2-hydroxypropyl)-methacrylamide
ACCP: activatable CPP
PLK1: polo-like kinase 1
Cop1: constitutional morphogenic protein 1
PSA: prostate specific antigen
PSMA: prostate specific membrane antigen
TPN: tumor-penetrating nanocomplex

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