Review

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Peptide-based vectors: recent developments

Abstract: Peptides and peptide-cargo complexes have been used for drug delivery and gene therapy. One of the most used delivery vectors are cell-penetrating peptides, due to their ability to be taken up by a variety of cell types and deliver a large variety of cargoes through the cell membrane with low cytotoxicity. *In vitro* and *in vivo* studies have shown their possibility and full effectiveness to deliver oligonucleotides, plasmid DNA, small interfering RNAs, antibodies, and drugs. We report in this review some of the latest strategies for peptide-mediated delivery of nucleic acids. It focuses on peptide-based vectors for therapeutic molecules and on nucleic acid delivery. In addition, we discuss recent applications and clinical trials.

Keywords: cell-penetrating peptide; delivery vector; nucleic acid; oligonucleotide delivery; peptide.

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Introduction

Peptide-based vectors are one class of biopolymers consisting of fewer than 50 amino acids connected by covalent peptide bonds. They have been studied for years due to their potential applications as a delivery system for many different types of therapeutic molecules. Cell-penetrating peptides (CPPs) are a class of short peptides, typically shorter than 30 amino acids, which unlike most peptides can cross the cellular membrane. The transcription-transactivating (Tat) protein of HIV-1 was the first CPP to be discovered more than 25 years ago. Frankel and Pabo (1) showed that Tat can enter cells and translocate into the nucleus. Since then, CPPs have been used for multifarious applications (2). Low cytotoxicity and the possibility to transport many different types of cargo are the two main characteristics that have made CPP applications of great interest for the delivery of therapeutic molecules. CPP strategies can be applied *in vitro* and *in vivo* (3, 4) for the delivery of therapeutic molecules, such as small interfering RNA (siRNA), plasmid DNA (pDNA), proteins, and other peptides.

Amino acid composition, hydrophobicity, and polarity are crucial for the three-dimensional (3D) structure of CPPs. The characteristics of the sequence (cationic, anionic, neutral, number, and order of amino acids) are important for the different uptake pathways (5). Furthermore, the different cell lines, types of cargo carried by a CPP, covalent or non-covalent attachment to the CPP, and concentration can influence cellular uptake efficiency and mechanism of internalization. Some of the most known CPPs are shown in Table 1. They can be divided and classified based on their origin (chimeric, synthetic, and protein derived) or sequence characteristics [positively charged, amphipathic (both cationic and anionic peptides), or hydrophobic CPPs].

Different strategies for peptide-mediated delivery

Covalent strategy

There are two ways to deliver cargo molecules with peptides: covalent conjugation and non-covalent complexation. The first strategy requires chemical synthesis of the modified peptide to facilitate the covalent conjugation of the cargo (18–21). Thioether, thiolmaleimide, ester formation, and click chemistry (Figure 1B) are some of the possible conjugation methods available (22, 23). One of the advantages of the covalent method is that the resulting peptide-cargo conjugate is a single entity, which is a desirable characteristic for drug design and *in vivo* applications. The main use of this strategy is for the delivery of DNA-mimic molecules or oligonucleotides such as peptide nucleic acids, phosphorodiamidate morpholino-oligomers (PMOs), peptides, and proteins. Several studies have been reported for peptides derived from Tat, the first CPP (1, 6, 24), for penetratin (7), and for polyarginine peptide Arg_8 sequence (11, 25).

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Table 1 Example of common CPPs.

Name	Sequence	Origin	Reference
$TAT_{(48–60)}$	GRKKRRQRRRQC	HIV-1 transcriptional activator	(6)
Penetratin	RQIKIWFQNRRMKWKK-NH_2	Antennapedia *Drosophila melanogaster*	(7)
pVEC	LLIILRRRIRKQAHAHSK-NH_2	Murine vascular endothelial cadherin	(8)
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL-NH_2	Galanin-mastoparan (chimeric)	(9)
Transportan 10	AGYLLGKINLKALAALAKKIL-NH_2		(10)
Polyarginine	R_n (n=6–12)	Model peptide (chimeric)	(11)
CADY	Ac-GLWRALWRLLRSLWRLLWRA-cya	Synthetic	(12)
PepFect3	Stearoyl-AGYLLGKINLKALAALAKKIL-NH_2	Synthetic analogue TP10	(13)
PepFect6	Stearoyl-AGYLLGK(K(K_2(tfq_4)))INLKALAALAKKIL-NH_2	Synthetic analogue TP10	(14)
MPG	Ac-GALFLGFLGAAGSTMGAWSQPKSKRKV-cya	SV40 NLS-HIV Gp41	(15)
KALA	WEAKLAKALAKALAKHLAKALAKALKACEA	Synthetic analogue HA_2 subunit of influenza hemaglutinin	(16)
Pep-1	KETWWETWWTEWSQPKKKRKV	Trp-rich motif-SV40 (chimeric)	(17)

cya, cysteamide; tfq, trifluoromethylquinoline moiety.

The delivery and cellular internalization of covalently linked cargos were improved by studying other protein-derived peptides such as pVec (8), antimicrobial peptides (26), and polyproline sweet arrow peptide (27). The limitation of this method is the possibility of affecting the biological activity of the cargo. This is the reason why the non-covalent strategy seems more appropriate for charged oligonucleotides, neutral molecules, or positively charged proteins.

Non-covalent strategy

The CPPs in this strategy consist of a polar (hydrophilic) domain and a non-polar (hydrophobic) domain. These short amphipathic peptides form non-covalent complexes with cargo molecules, thereby enhancing the delivery into cells. The primary and/or secondary structures are accountable for the amphipathic character of the peptide. Primary amphipathic peptides have a sequential assembly of hydrophobic and hydrophilic residues. Secondary amphipathic peptides are formed by the conformational state that allows the positioning of hydrophobic and hydrophilic residues on opposite sides of the molecule (28). Stable complexes between oligonucleotide and peptide are formed by non-covalent interactions (electrostatic and hydrophobic). Examples of primary amphipathic peptides are Pep-1 and MPG (Table 1). The first one was used for the delivery of small peptides and proteins (17), whereas the second one was used for the delivery of siRNA (29).

The non-covalent strategy was used to complex peptide with negatively nucleic acids, and it was originally developed for gene delivery and later on for protein and oligonucleotide delivery, using also other CPPs such as Tat (30), polyarginine (31, 32), and Transportan-derived peptides (33, 34).

Mechanisms of uptake

It is crucial to understand the cellular uptake mechanisms of CPP with cargo molecule, especially for the development and optimization of appropriate strategies for therapeutic applications. The first studies on cellular internalization of CPPs reported that their mechanism of uptake was independent of endocytosis and does not use energy or a specific receptor. Over the last few years, the mechanism of many CPPs has been re-examined and reported to be mediated by endocytosis. The major mechanisms used by CPPs to pass the cellular membrane and enter into the cell are endocytosis and direct translocation (35). Peptides with strong affinity for the cell membrane can enter the cell through an endocytic pathway due to the internalization and recycling processes of the cell surface. Endocytosis occurs by various mechanisms, which can be divided into clathrin-dependent endocytosis (CDE) and clathrin-independent endocytosis (CDI). In CDE, the cytoplasmic domains of plasma membrane proteins are recognized by adaptor proteins and packaged into clathrin-coated vesicles that are brought into the cell (36). CDI comes in many forms, such as macropinocytosis and caveolae and/or lipid raft-mediated endocytosis (37). All these different pathways are involved in the uptake of CPPs (Figure 1C).

Uptake is dependent on cell membrane composition, which includes density, fluidity, and different lipid compositions (38). The cell lines used for the studies are crucial for the uptake mechanism and levels. The physicochemical properties of a CPP conjugate with a cargo molecule are important for direct penetration of the cell membrane. This possible route of uptake for complexes is a similar to the uptake mechanism for small molecules.

There are studies showing that the mode of uptake of many CPPs, including Tat-derived peptides, R9, and

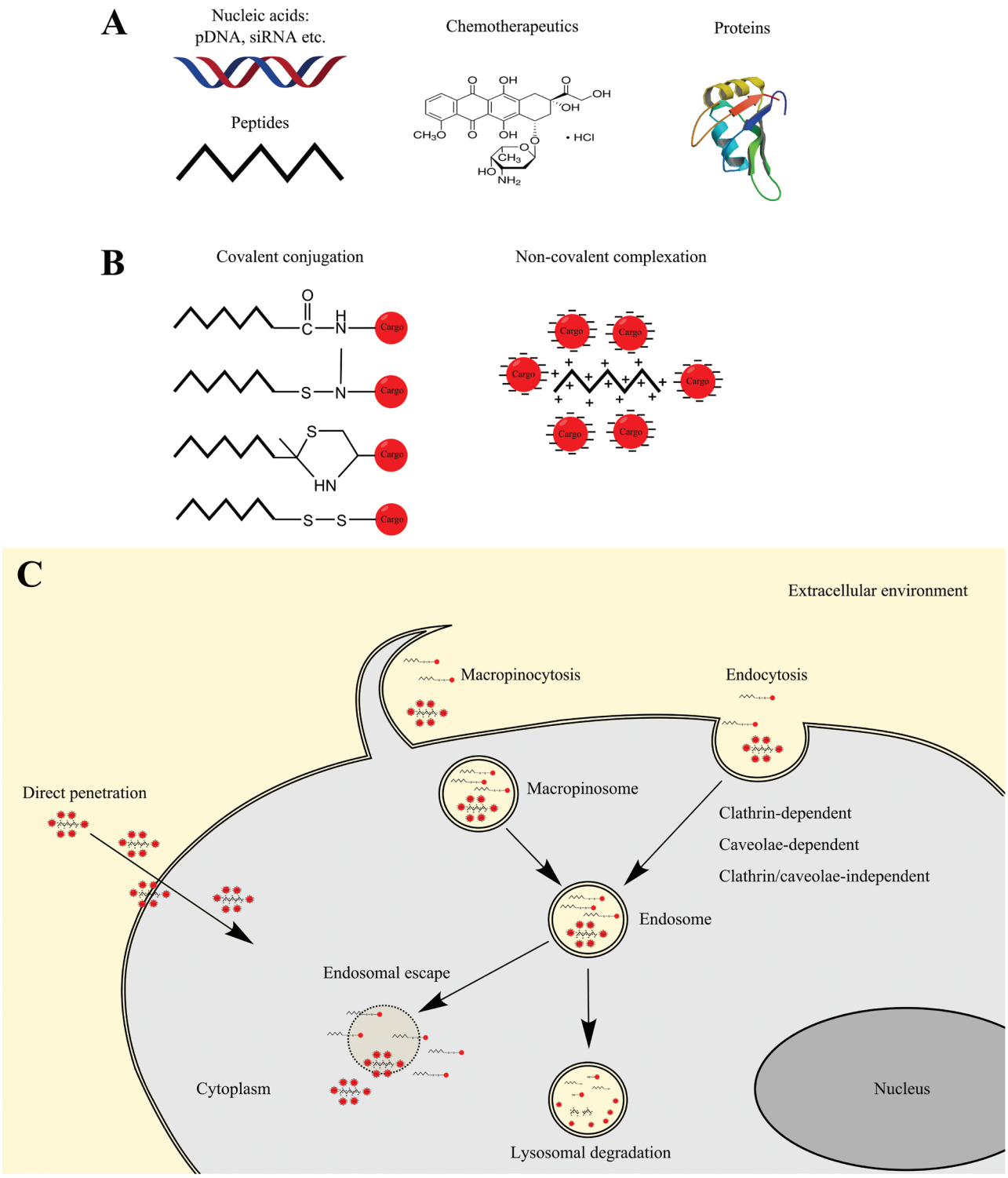

Figure 1 Different strategies for peptide-mediated delivery and mechanisms of internalization of CCPs.
Peptide and different possible cargo (A). Covalent and non-covalent strategies for conjugation/complexation of CPP with cargo molecule (B). CPPs or CPP/cargo complexes bind to extracellular matrix via the proteoglycan on the cell surface, increase of membrane fluidity or microdomain dynamic promotes the cell entry and release in the cytosol of CPP non-covalently complexed and of CPP covalently conjugated via membrane fusion or cellular uptake of CPP via endocytosis pathway (•clathrin-dependent, •caveolae-dependent, • clathrin- and caveolae-independent) or macropinocytosis. After endocytic capture, CPPs can escape from lysosomal degradation and enter the cytosol and the nucleus, remain in the endosomes, or be delivered in the Golgi apparatus and the endoplasmic reticulum (C).

penetratin (39–41), depends on CPP concentration. Usually, CPP concentration is in the micromolar range, even if the concentration threshold can be different and depends on CPP. However, the cellular uptake mechanism remains controversial and still needs to be confirmed for many CPPs. One of the reasons why the cellular uptake mechanism should be confirmed is because the use of fluorescein-labeled CPPs for the visualization of CPPs inside the cell poses a risk of altering the uptake mechanism or activating another cell entry pathway. Despite the controversy, a general scheme for the CPP uptake mechanism is accepted where the proteoglycans play an important role in the electrostatic interactions between the CPP and the cell surface. It is important to study the uptake mechanism and the biological effects of the complexes instead of the peptide alone. In 2012, the involvement of scavenger receptor class A (SCARA) in the uptake of noncovalent CPP:ON complexes was shown for the first time (42). It was previously shown that SCARA receptors bind and mediate cellular uptake of a negatively charged molecules (43), and the involvement of SCARA was shown for the CPP PepFect14 and splice-correcting oligonucleotides (SCOs), which have negative ζ potential and NF51/pDNA (44). The nature and secondary structure of the CPP, the ability to interact with cell surface and membrane lipid components, the nature, type, and active concentration of the cargo, the cell type, and the membrane composition are the parameters that play a secondary role in the cellular uptake pathway (Figure 1C).

Applications and clinical trials

CPPs have not yet become pharmaceutical products on a large scale, nor have peptides in general. The main two obstacles are the duration of action and the way of administration. The first drawback is due to proteolysis and clearance (both renal and liver clearance), and a possible solution might be the use of unnatural amino acids. The second drawback can be solved by a new formulation that uses a different route of administration such as oral, intranasal, and inhalation routes. More studies and efforts about the synthesis, formulation, toxicology, delivery, and clinical studies of peptides can lead to valid therapeutic peptide-based drugs. Only a few CPPs are currently in clinical trial, including AZX100 (Capstone Therapeutics, http://www.capstonethx.com/, keloid scarring, phase II), RT001 (ReVance Therapeutics, http://www.revance.com/, wrinkling of the skin, phase II), KAI-9803 (KAI Pharmaceuticals, http://www.kaipharma.com/, myocardial infarction, phase II), and XG-102 (Auris Medical, http://www.aurismedical.com/, hearing loss, phase II). DTS-108 (Diatos, cancer) is in preclinical studies (Table 2).

Delivery of genetic material

Delivery of pDNA

pDNA is a classical tool in gene therapy because it allows the restoration/replacement of functioning/malfunctioning gene expression levels, respectively. The native form of the gene is incorporated into the plasmid, which is then included into the carrier system with proper targeting and pharmacokinetic properties (45). The main hurdle for pDNA-based gene therapy is the problem in reaching the cell nucleus for the gene to get expressed, which is an additional rate-limiting step, compared with RNA interference (RNAi) therapeutics, which only have to reach the cytoplasm to be functional. Until now, there have not been many successful reports for systemic pDNA delivery *in vivo* with peptide-based delivery vectors. This is mainly due to inefficient condensation of pDNA by peptide vectors into nano-sized complexes and poor endosomal escaping properties of these complexes, leaving them therapeutically less potent (46). Furthermore, the de-complexation of carrier from pDNA is very important, and the 3D structure of pDNA has to recover in the nucleus to be biologically active (45).

Table 2 Summary of clinical trials.

Name	Company	Phase	Disease	Reference
p28	Pediatric Brain Tumor Consortium	I	Recurrent/progressive CNS tumors	http://clinicaltrials.gov/
DTS-108	Bio Space-Diatos	I	Carcinoma	http://www.draispharma.com/
AZX100	Capstone Therapeutics	II	Dermal/keloid scarring	http://www.capstonethx.com/
KAI-9803	KAI Pharmaceuticals	II	Myocardial infarction	http://www.kaipharma.com/
XG-102	Auris Medical	II	Hearing loss	http://www.aurismedical.com/
RT001	ReVance Therapeutics	III	Lateral canthal lines	http://www.revance.com/

For both pDNA and short oligonucleotide delivery, physicochemical properties such as size, size distribution, and the ζ potential of the formed nanoparticles with transport vectors are very important because they roughly define the destiny of the nanoparticles in the organism. For example, smaller particles are excreted from the body by renal clearance and larger particles, >300 nm, tend to get stuck in the lungs, which sometimes causes acute toxicity (47). Also, the surface charge of the nanoparticles plays a key role in their destiny because particles with larger ζ potential are more easily recognized by opsonins and are consequently picked up by liver macrophages. It was hypothesized that the ζ potential of nanoparticles should be between -30 and 30 mV to reduce interactions with blood components and applicable colloidal stability, as the colloidal stability increases together with ζ potential, making it too difficult for the cargo to de-complex from the nanoparticles in the site of action. Furthermore, the size distribution of the nanoparticles, also known as the polydispersity index (PDI), is important because it describes the quality of the measured dispersion. Usually, the size of dispersion is given as average with standard deviation, which does not show if the dispersion has many size fractions, for example.

Considering the importance of size distribution, Dash et al. (48) used elastin-like polypeptides (ELPs) (Table 3) to obtain monodispersed hollow spheres for pDNA encapsulation and delivery. A template-based method was used, where sulfonated monodispersed polystyrene beads with negative surface charge were covered with ELPs with slightly positive charge, which were further cross-linked with transglutaminase treatment. After that, polystyrene beads were dissolved with tetrahydrofuran and hollow spheres were obtained. Hollow spheres measuring 300 nm showed 54% encapsulation efficiency for naked pDNA, compared with 98% for pDNA in polyplexes, whereas polyplex-loaded hollow spheres had higher delivery efficiency and lower toxicity than pDNA polyplexes in human adipose-derived stem cells (ADSC) and human umbilical vein endothelial cells (HUVEC) and had 10 times higher luciferase expression than naked pDNA-loaded spheres.

Upon systemic administration of any kind of nano-sized drug formulations, it is important to increase the specificity toward the tissue or cells that need treatment to reduce dose-related side-effects. One way to add specificity to delivery formulations is to modify its surface with targeting molecules (e.g., folic acid) or peptides (tumor-homing peptides).

Zhang et al. (49) developed a multifunctional peptide-PEG-Tris-acridine conjugate (pPAC) that intercalates with pDNA, allowing PEG-shielding capability and peptide delivery and targeting passage through the blood-brain barrier. Unfortunately, these pPACs were not able to condense pDNA, and therefore, for sufficient polyplex formation, they added linear PEI derivatives to the mix. For the target gene, they chose multi-drug resistance protein 4 (MRP4) for which a plasmid vector expressing anti-MRP4 shRNA was constructed. To achieve better vectorization to the brain, pPACs were decorated with brain-homing (BH) or apolipoprotein E (ApoE) (Table 3) receptor-specific peptides, which both showed similar efficiency when the amount of peptide in formulations was increased. One hour after intravenous injection in the tail vein of Balb/c mice, brain accumulation increased up to 3-fold over non-targeted formulation. They further confirmed that these polyplexes were delivered into the brain vasculature. Finally, they showed that targeted knockdown of MRP4 increased the accumulation of azidothymidine, which is used in antiretroviral AIDS treatment, in the brain by up to 3.7-fold.

Table 3 Summary of described peptides-based delivery vectors for genetic material.

Name	ON cargo	Target/cargo type	Administration route(s) and dosing in mice	Reference
PepFect3	pDNA	Luciferase	i.m.; 10 μg/mouse once	(63)
ELP	pDNA	Luciferase	*In vitro*	(48)
ApoE (targeting)	pDNA coding shRNA	MRP4	i.v.; 20 μg/mouse on days 1, 3, and 5	(49)
dTAT	pDNA	Ang II	Intratracheal injection; 0.7 μg/mouse on days 3, 7, 10, and 14	(50)
NAG-MLP	siRNA	Factor VII, HBV	i.v.; 2 mg/kg for F7 and 3 mg/kg for HBV once	(53)
PepFect6	siRNA	Luciferase, HPRT1	i.v.; 1 mg/kg for HPRT1	(14)
cKK-E12	siRNA	Factor VII	i.v.; 0.03 mg/kg once	(54)
PVBLG-8	siRNA	T-oligo	i.v.; ~10 mg/kg daily for 3 weeks	(55)
$(RXRRBR)_2XB$ peptide	SCO	A-T	i.v.; 60 mg/kg daily for 4 days	(57)
B-MSP	SCO	Dystrophin	i.v.; 3 and 6 mg/kg biweekly for 12 weeks	(58, 59)
Pip5e	SCO	Dystrophin	i.v.; 25 mg/kg once	(61)
Pip6 peptides	SCO	Dystrophin	i.v.; 12.5 mg/kg once	(60,62)

i.m., intramuscular; i.v., intravenous; MRP4, multi-drug resistance protein 4; Ang II, angiotensin II; HBV, hepatitis B virus; HPRT1, hypoxanthine phosphoribosyltransferase 1.

To avoid size-related toxicity during systemic administration for some diseases, alternative administration routes can be used. Kawabata et al. (50) showed that the TAT analogue, with two TATs connected in tandem (dTAT), was able to mediate efficient angiotensin II (Ang II) coding pDNA (pAT2R) delivery into Lewis lung cancer (LLC)-bearing mice lungs after intratracheal administration with an intratracheal sprayer. dTAT/pAT2R non-covalent complexes significantly attenuated the growth of fast-growing LLC tumors, showing to be a very promising gene delivery method for lung cancer treatment (50).

Delivery of oligonucleotides

siRNA delivery holds a promising possibility in the down-regulation of over-expressed or defective genes through endogenous post-transcriptional gene silencing also known as RNAi (51). Therapeutic applications of siRNA range from viral infection treatments to hereditary disorder and cancer treatments (52); therefore, they can be found in quite many clinical trials already. To be an effective systemic delivery, RNAi therapeutics have to overcome many biological obstacles, such as degradation by nucleases and upon reaching the cytoplasm, siRNAs have to be included into the RNAi machinery to carry out their biological effect.

Nanoparticles mainly end up in scavenger organs like liver, kidneys, and spleen due to their treatment as foreign bodies by the organism. In the previous decades, a lot of work has been put into delivering siRNAs to the liver, which has led to the first clinical trials on vectorized siRNA delivery. Few recent successes have been described below.

Wooddell et al. (53) developed a hepatocyte-targeted delivery system for the treatment of chronic hepatitis B virus infection (HBV). For the systemic delivery, they used *N*-acetylgalactosamine-conjugated mellitin-like peptide (NAG-MLP) with liver-tropic cholesterol-conjugated siRNA (Chol-siRNA). A dose of 2 mg/kg of Chol-siRNA was required for maximal (>95%) knockdown of coagulation factor VII (F7) in the liver, which was over 2-fold lower in non-human primates than in mice. For the HBV treatment study, they used HBV-infected NOD-SCID mice and transgenic HBV mice. Mice were co-injected intravenously with Chol-siHBV and NAG-MLP, showing efficient knockdown levels of HBV mRNA, protein, and viral DNA at Chol-siRNA doses of 1 mg/kg in infected mice and 3 mg/kg in transgenic mice at NAG-MLP dose of 6 mg/kg in both cases.

Recently, our group developed a CPP designed to enhance endosomal release of the siRNA into the cytosol. The described CPP, PepFect6, has a peptide backbone of a parent peptide transportan 10 (TP10) with N-terminal stearic acid modification (also known as PepFect3) (Table 3) for enhanced serum stability. Additionally, four pH titratable trifluoromethylquinoline moieties were added on a lysine-tree attached to the Lys^7 ε-amino group of the peptide to increase endosomal escape. It is known that buffering of the endosomal pH causes osmotic swelling and consequent rupturing of the endosomal membrane, releasing the cargo inside. After systemic administration, the PepFect6/siRNA complexes induced up to 60% silencing of the endogenous housekeeper gene HPRT1 in the lungs and kidneys and even more in the liver at a dose of 1 mg/kg of siRNA without any observable toxicity (14).

In another study inspired by lipoproteins, Dong et al. (54) synthesized a library of lipopeptides that were incorporated into lipid nanoparticles, forming a delivery system called lipopeptide nanoparticles (LPNs) designed for *in vivo* siRNA delivery into liver hepatocytes. The most efficient lipopeptides consisted of dilysine-based diketopiperizines, which were reacted with 1,2-epoxydodecane to yield lipopeptide with four fatty acids, and the most potent was cKK-E12. LPNs with cKK-E12 were able to induce maximal (>95%) coagulation factor VII (F7) knockdown at siRNA doses of 0.03 mg/kg in mice and 0.3 mg/kg in non-human primates. Toxicity studies carried out with cKK-E12 LPNs on rats did not show any toxicity at siRNA doses as high as 1 mg/kg, which makes it one of the most efficacious delivery systems yet reported.

As an alternative to liver delivery, Uppada et al. (55) used oligonucleotides homologous to 3′-telomere overhangs (T-oligos), which consist of 11 bases. These T-oligos were used for melanoma therapy in a xenograft model. T-oligos mimic the exposure of the 3′-telomere overhangs, inducing DNA damage response signals in several cancers that are otherwise bypassed in tumor cells, therefore reducing tumorigenicity and metastasis by causing biological aging, reduced vascularization, and apoptosis. In the study, 0.52 nmol (approx. 0.2 mg/mouse) of T-oligos complexed with cationic α-helical peptide PVBLG-8 (PVBLG) (Table 3) resulted in 9-fold reduction of tumor volume after daily intravenous injection compared with diluent treatment. The treatment was started 1 day after the induction of the xenograft tumor, and mice were treated for 3 weeks. Although this method shows promising tumor-specific effects, the efficiency and specificity of this delivery platform has to be further optimized.

Delivery of SCOs

Another antisense-based method besides siRNAs are single-stranded SCOs, which act in the cell nucleus as pDNA

as a means of delivery. SCOs redirect splicing by attaching to the pre-mRNA, allowing the masking of either the normal or aberrant splicing sites, which leads to exon skipping or exon inclusion, respectively (56). Currently, the most studied therapeutic applications for SCOs are neurodegenerative diseases such as muscular dystrophy where mutation in the exon causes dysfunctional dystrophin protein expression. In addition, SCOs can be easily chemically modified to increase serum stability. For example, the phosphate backbone of SCOs can be chemically modified, so it becomes uncharged, as it is for PMO. This allows covalent conjugation of positively charged transport vectors such as CPPs, which would otherwise lead to aggregation of conjugates.

In a study by Du et al. (57), the arginine-rich peptide $(RXRRBR)_2XB$ (Table 3) was used for covalent splice-correcting PMO delivery against the ataxia-telangiectasia (A-T) mutated (ATM) gene. A-T is an inherited progressive neurodegenerative disease known to cause movement and coordination problems and immunodeficiency. ATM protein also plays a key role in recognizing DNA damage, thus additionally increasing the risk of cancer. In some studies where lymphoblastoid cells derived from patients with A-T were used, it was shown that the $(RXRRBR)_2XB$-PMO conjugates were able to almost fully correct the aberrant splicing with sustained ATM protein levels up to 21 days. Intravenous delivery of 60 mg/kg of fluorescently labeled $(RXRRBR)_2XB$-PMO conjugates showed uptake in the brain, which was more profound in all areas of the brain after daily treatment for 4 days. Single treatment showed localization in GABAergic neuronal Purkinje cells, which play a critical role in A-T pathogenesis, and multiple treatment further increased localization in the cerebellum and Purkinje cells (57), showing potential for this conjugate in brain delivery.

In another study, Yin et al. (58) further developed their previously published muscle targeting B-MSP-PMO delivery platform (59) for the treatment of Duchenne muscular dystrophy (DMD), where B is the arginine-rich $(RXRRBR)_2$ peptide, which is fused to muscle-targeting heptapeptide (MSP) sequence ASSLNIAX and conjugated to PMO. After intravenous administration of B-MSP-PMO conjugate at low doses, 3 and 6 mg/kg injected two times per week for 12 weeks, it showed 100% dystrophin-positive fibers and almost complete splice correction in peripheral muscle groups in *mdx* mice (Table 3). The consequent dystrophin protein level restoration was shown to be 50% after 12 weeks without any noticeable toxicity or immune responses, which clearly implicates the therapeutic potential of described delivery platform.

In a study based on previous results, Betts and colleagues (60) developed a new generation of Pip6-PMO conjugates with the aim to increase the cardiac exon skipping in DMD treatment compared with their previous lead conjugate Pip5e-PMO, with the peptide sequence RXRRBRRXRILFQYRXRBRXRB. Upon intravenous administration of 12.5 mg/kg Pip6-PMO conjugate in mdx mice, it became evident that Pip6a (ILFQY core inverted), Pip6b (core changed to IQFLI), and Pip6f-PMOs (core scrambled FQILY) have increased dystrophin production activities compared with the parent compound (Table 3). Although these conjugates were more efficient than the previous lead peptide, they were still not optimal peptides for the delivery of PMOs and their efficiency remains to be studied (61). Further, mechanism studies with Pip6a-PMO show that it is taken up by energy-dependent and caveolae-mediated endocytosis in the skeletal muscle cells. Also, in primary cardiomyocytes, it mainly ends up in cytoplasmic vesicles where CDE is dominant. This explains why Pip6a-PMO has lower activity in myoblast and cardiomyocytes than in myotubes and thus having lower dystrophin restoration activity in heart muscle tissue than in skeletal muscle tissue, respectively (62).

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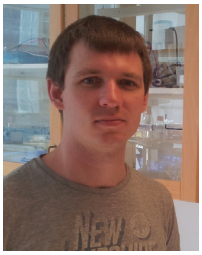

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