

Review

# Peptide-Based Vectors for Gene Delivery

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**Abstract:** Gene therapy is the ultimate therapeutic technology for diseases related to gene abnormality. However, the use of DNA alone has serious problems, such as poor stability and difficulty in entering target cells. The development of a safe and efficient gene delivery system is the cornerstone of gene therapy. Of particular interest, multifunctional peptides are rationally designed as non-viral vectors for efficient gene delivery. As components of gene delivery vectors, these peptides play critically important roles in skeleton construction, the implementation of targeting strategies, cell membrane penetration, endosome rupture, and nuclear transport. In recent years, the research of functional peptide-based gene delivery vectors has made important progress in improving transfection efficiency. The latest research progress and future development direction of peptide-based gene delivery vectors are reviewed in this paper.

**Keywords:** gene delivery vector; gene therapy; non-viral vector; functional peptides

## 1. Introduction

Gene therapy, which relies on gene replacement to correct or compensate for gene defects or abnormalities, has emerged as a powerful therapeutic approach to treat diseases, such as cancer diseases and monogenic diseases [1–5]. In practice, if DNA is injected into animals via intravenous injection, the treatment efficiency is generally very low [6,7]. This is because after entering the body, the therapeutic gene is easily degraded by the nucleases in the serum. In addition, a gene generally has a relatively large molecular mass with a negative charge, which makes it difficult to adhere to and pass through the negatively charged cell membrane. Therefore, the safe transportation of therapeutic genes into target cells and efficient expression are the keys to the success of gene therapy, which heavily relies on promising gene delivery systems with a high transfection efficiency, low toxicity, specific targeting, good stability, and easy mass production [8,9].

There are two types of gene delivery vectors: viral vectors and non-viral vectors [10]. Viral vectors utilize the inherent ability of wild-type viruses so that they can highly infect cells. The viral vector is the first vector used to protect and transmit genes. Common viral vectors include adeno-associated virus, lentivirus, and retrovirus [11–13]. The advantages of viral vectors are that they can carry genes to efficiently transfect various types of human cells, even non-dividing cells, and have very high gene transfection efficiency [14]. However, a viral vector has limitations on the size of nucleic acid molecules that can be carried and requires a complex preparation process, which is high in cost. More importantly, it has potential safety hazards such as immunogenicity and carcinogenicity [15]. These defects greatly limit the practical application of viral vectors. On the contrary, non-viral vectors have been widely developed and paved multiple cornerstones in the field of gene delivery. Various non-viral vectors, such as cationic liposomes, polymers, peptides, dendritic cations, and inorganic nanoparticles (e.g., mesoporous materials and quantum dots) have been employed for the delivery of genes [16–19]. Compared with viral vectors, these non-viral vectors have higher safety and are feasible to integrate multiple functionalities for



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overcoming diverse biological barriers that are encountered in the process of gene delivery. In recent years, the number of non-viral vectors entering clinical trials has been gradually increasing [20].

Peptides have been regarded as perfect building blocks for non-viral vectors owing to their unique advantages, such as good biocompatibility, functional diversity (such as membrane permeability, targeting, and endosome escape), biodegradability, and ease of synthesis and modification [21,22]. Short cationic peptides can be used alone or as fusion proteins to bond and condense appropriate DNA, resulting in the formation of gene-loaded peptide-included complexes with prospective functionalities for targeted cell internalization and efficient intracellular transfection [23–25]. To date, various peptide-based gene delivery systems, such as virus-mimicking peptide particles, hybrid peptide systems assisted by lipids or polymers, and polypeptide nanostructures, have been constructed for the purpose of organelle targeting, molecular imaging, and anticancer therapy [26–28]. In this review, we will highlight the significance of peptide-based gene delivery systems as potential therapeutic nanomedicine for the treatment of diseases.

## 2. Plasmid DNA (pDNA)

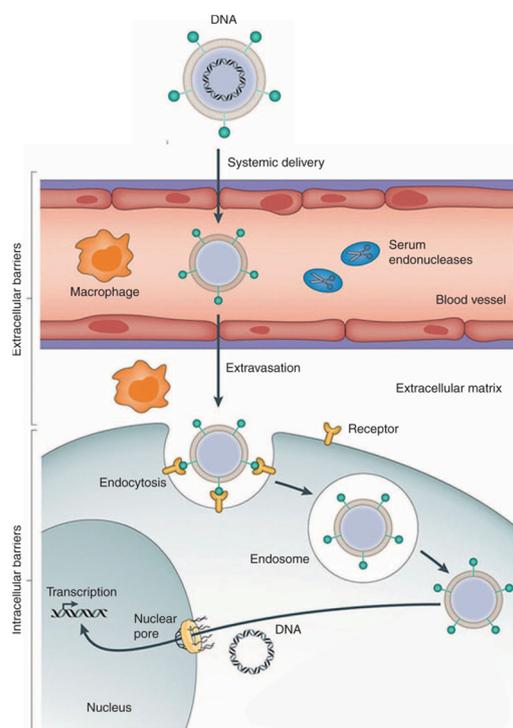
When the target gene with therapeutic effect and all cis-regulatory elements, such as a promoter, enhancer and silencing sequence and transcription processing signal, is inserted into the plasmid, it can be used for the transfection of mammalian cells. When a virus replicon is contained, pDNAs can be guided to amplification in the target cell nucleus. Most pDNAs are circular double-stranded DNA, which exist in the cytoplasm and can replicate by themselves in target cells [29]. They are intracellular genetic materials that are independent of chromosomes. Importantly, pDNAs have become an extremely important class of drugs in gene therapy, and they are most widely used because of their advantages of easy production, low immunogenicity, safety, good stability, and convenient use. Since 1995, pDNAs have been used in approximately 25% of approved gene therapy trials [30]. However, pDNAs are usually large in molecular size, which adds some difficulties to their delivery into cells.

## 3. Delivery Barriers of Gene Therapy

During the transportation of DNA with the aid of synthetic vectors, there are multiple barriers that need to be surmounted for efficient gene delivery, which usually depends on the types of genes (DNA or RNA) and cells.

As shown in Figure 1, the general process of gene delivery mainly includes systemic delivery through blood circulation, extravasation into diseased tissue, specific cell targeting, cellular penetration, endosome escape, and, sometimes, nuclear import [31]. During this process, the gene delivery system would confront diverse biological barriers until it finally reaches the action site. First, high stability and efficient packaging/condensation are the prerequisites for long-term blood circulation, which is critically important for adequate DNA to reach the diseased lesions [32]. Specifically, DNA should be loaded into a compact vector that relies on the electrostatic interaction between negatively charged genes and positively charged carriers to facilitate efficient delivery. As a result, the obtained gene delivery vectors would possess negative or positive charges, which exhibit different biological behaviors, such as the efficient cellular uptake of positively charged complexes, while it brings high cytotoxicity and probable aggregation in physiological fluids as well. Therefore, a suitable gene-to-carrier ratio would be the key to efficient gene loading and transfection, which minimizes cytotoxicity. Also, polyethylene glycol modification, the so-called PEGylation, connects the PEG to the carrier through chemical bonds. The PEG is a charge-neutral polymer, which is the most commonly used stabilizer. PEGylation was proposed as an effective approach to form a stealth outer layer of the vectors for reducing aggregation and nonspecific interactions with polyanionic biomolecules in the bloodstream so as to avoid rapid clearance by the reticuloendothelial systems and extend the blood circulation time [33–35]. Second, the vector–DNA complexes must make contact with the cell

membrane before being ingested by the cells [36]. Complexes enter cells via endocytosis, which is usually mediated by a charge interaction with the cell membranes or by active targeting via receptor-mediated endocytosis. To maximize the targeted cell internalization and reduce nonspecific cell uptake in undesired cells, the gene delivery vectors are usually additionally modified with targeting groups, such as monoclonal antibodies, transferrin, folate, and peptides, which can bond to specific receptors that are overexpressed on particular diseased cells [37–39]. Third, after cell attachment, the vector–DNA complexes are encapsulated in the endosome and then transported to the lysosome, which possesses a highly acidic environment. Therefore, the vectors need to escape rapidly from the endo/lysosome into the cytoplasm; otherwise, low pH and enzymes in the endo/lysosomes would degrade the DNA or vector [40]. To promote endo/lysosomal escape, numerous methods have been proposed, including the proton sponge effect of cationic polymers (e.g., polyethylenimine (PEI)), photochemical internalization, and cell-penetrating peptides (CPPs) that facilitate endosomal release [41–44]. Forth, after escaping from the endo/lysosome, the vector–DNA complexes need to diffuse into the cytoplasm, and DNA should be unpacked from the condensed carriers as free molecules for reaching and entering the nucleus. At this step, the nuclear envelop would be the most difficult barrier, which separates the nucleus from the cytoplasm and includes nuclear pore complexes with a diameter of ~10 nm [45]. As such, the structure of the nuclear envelop is too preventive. Only small molecules (less than 50 kDa) can freely enter and exit the nucleus, but this is not conducive to the entry of DNA or vector–DNA complexes, which severely restricts the expression of DNA [46]. This theory has been supported by some imaging evidence in recent years. Collectively, the design of gene delivery systems should consider all the issues mentioned above that include but are not limited to DNA, diverse genetic disease (e.g., cancer and hypercholesterolemia), multiple functionalities (e.g., stealth, targeting, endo/lysosome escape, and nuclear import), and administration routes (e.g., subcutaneous and intramuscular administration and oral routes).



**Figure 1.** Biological barriers involved in gene delivery. Adapted with permission from the authors of [43].

## 4. Peptide-Based Gene Delivery Vectors

### 4.1. Peptides Act as DNA-Binding Units

Peptide-based vectors are mainly cationic peptides that are rich in basic amino acids (e.g., lysine, arginine, and histidine), which are positively charged at a normal physiological pH and can form complexes with negatively charged DNA [47]. Therefore, peptide-based gene delivery vectors can compress DNA, reduce their molecular size, and provide sufficient protection against nucleases. Meanwhile, the extra positive charge on the surface of the complexes can make it more accessible to the negatively charged cell membrane and promote complexes to enter the cells. For peptide-based vectors, at least 6~8 cationic residues are needed to effectively encapsulate and successfully transfect DNA, which depends on the amino acid category. For example, arginine binds DNA more strongly than lysine.

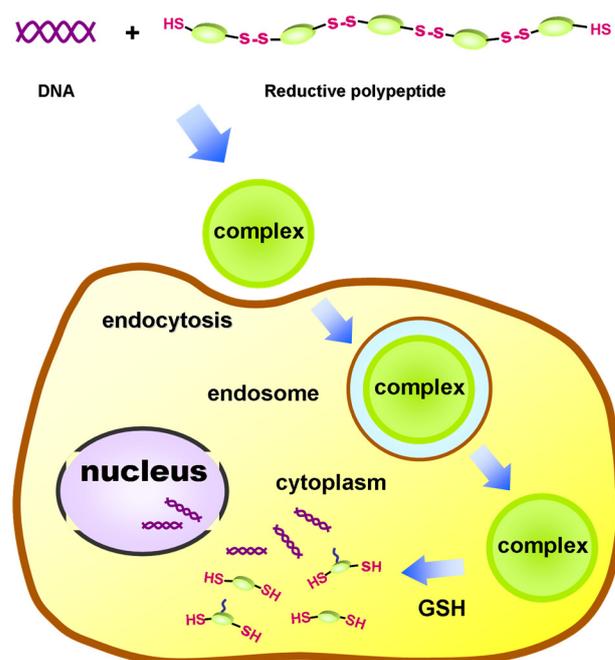
#### 4.1.1. Poly-l-lysines (PLLs)

A PLL is the first cationic peptide mediating gene transfer, and it is a linear polypeptide formed by polymerizing 90~450 lysine monomers. A PLL contains a primary amine, which is positively charged after protonation [48]. Thus, it can be used for gene transfer via electrostatic binding to negatively charged DNA. The compress ability of PLL to DNA enhances with the increase in molecular weight, and only PLLs with a molecular weight greater than 3000 Da can compress DNA to form stable complexes. PLLs have been widely used in gene delivery because their structures are easy to modify and can introduce various functional molecules. However, the transfection efficiency of PLLs is relatively low because (1) PLLs have a certain extent of cytotoxicity. A PLL's ability to compress DNA increases with its molecular weight, and higher-molecular-weight PLLs have good compression ability, which leads to greater cytotoxicity. (2) PLLs alone lack the ability to target specific cells and to escape from endosomes. At a physiological PH, the primary amines of PLLs are all protonated, resulting in their lack of buffering capacity in the acidic environment of endosomes after entering into cells. It is difficult for the PLL/DNA complex to escape from the endosome, which may eventually lead to DNA degradation by the nuclease. (3) The stability of the complexes is poor, and they easily form aggregates with the serum owing to the excess of positive charges [49–51].

A significant advantage of PLLs lies in their ease of chemical modification, and researchers have modified and improved the properties of PLLs in a variety of ways [52]. In order to reduce the cytotoxicity of PLLs, researchers began to modify them with the shielding polymer, PEG. In the work reported by Kim et al., they found that PEG modified on the amino group of a PLL could effectively reduce toxicity [53]. This method could reduce the binding of gene nanoparticles to serum in cells and increase the blood circulation time. Also, Kataoka et al. found that the particle size of the complex particles formed by the PLL-PEG copolymer and antisense-ODN was significantly lower than that of PLLs [54]. Their particle size distribution was extremely narrow, and the resultant core-shell architecture could resist deoxyribonuclease degradation. To further reduce undesirable side effects, Zhang et al. designed ternary FK/p53/PEG-PLL(DA) complexes by introducing charge-reversible dimethylmaleic anhydride (DA) groups in addition to PEG, resulting in the formation of a detachable shielding layer (PEG-PLL(DA)) on the nanocomplex surface [38]. At a physiological pH of 7.4, the FK/p53/PEG-PLL(DA) complexes could extend the circulating time due to the charge shielding effect of the PEG-PLL(DA). Once it arrives at the acidic microenvironment of the tumor tissue, the PEG-PLL(DA) undergoes a charge switch for the removal of the surface shielding layer, which facilitates the subsequent tumor-cell-specific targeting. Moreover, Guo et al. covalently grafted a sugar-containing polymer (DPMAEL) onto PLLs, which not only reduced the cytotoxicity of the PLLs, but also improved the stability of the complex nanoparticles in the electrolytes and serum [55]. In order to improve the escape ability of the endosome and the transfection efficiency of the PLLs, Longer et al. grafted the amino group in the side chain of PLLs with the imidazole group, and the corresponding gene transfection efficiency was significantly improved [56].

An analysis showed that the grafted imidazole group was easily protonated in an acidic environment, and the electrostatic attraction with DNA was enhanced, which, at the same time, enhanced the buffer capacity of PLLs for efficient endo/lysosome escape. Kim et al. reported that the PLL linked with 16% histidine was a highly efficient transgenic vector with little toxicity [57]. Midoux et al. found that the transfection efficiency of partially histidylated PLLs increased without the assistance of chloroquine. This was related to the fact that histidylated PLLs could be further protonated at a low pH, which partially mimicked the proton sponge mechanism of PEI [58].

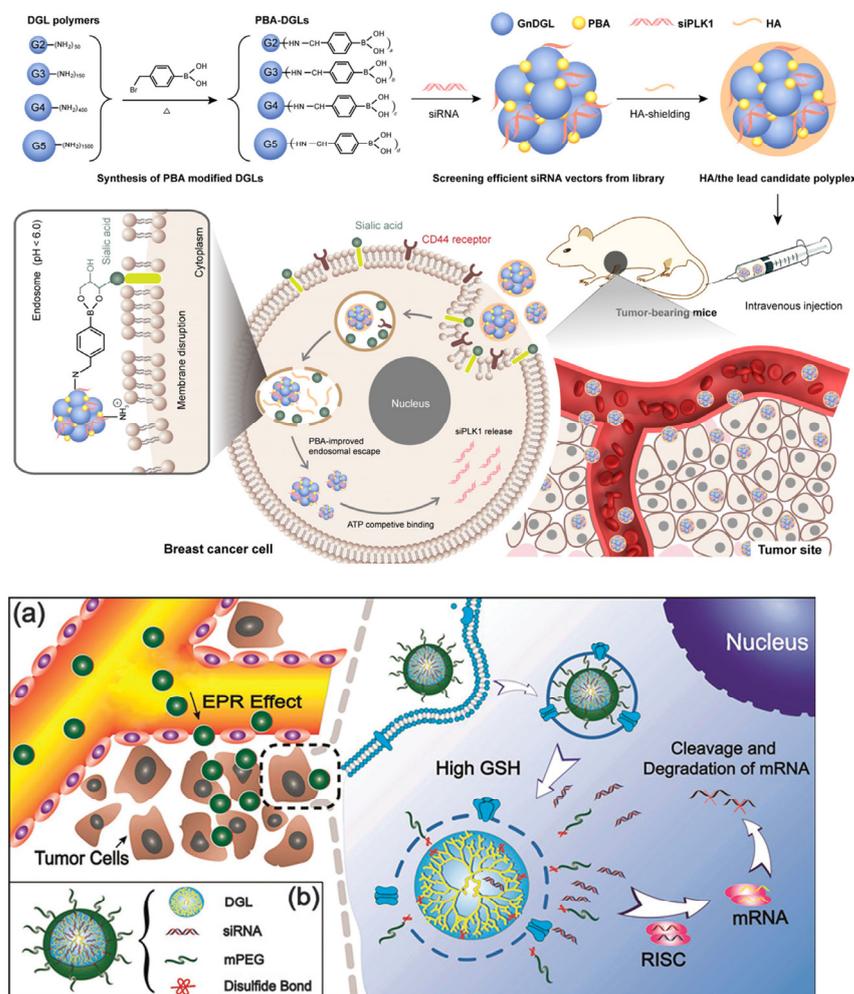
A common issue faced by polycation vectors is how to strike a balance between toxicity and transfection efficiency. The introduction of a reduction-responsive group into the carrier by molecular design is a viable strategy, such as using a disulfide-crosslinked carrier [59–61]. The disulfide bonds can be cleaved in the highly reducing environment in cells, which contributes to the release of nucleic acid molecules into the cytoplasm. As an example, Zhang et al. constructed a PEGylated reductive vector by oxidatively polycondensing  $\text{CHK}_6\text{HC-mPEG}$  and  $\text{CHK}_6\text{HC}$ , both of which contained the short chain of lysine segments, via disulfide bonds (Figure 2) [62]. The disulfide bonds in the resultant nanovectors were reductively degraded into short-chain oligopolylysine in an intracellular environment, and the cytotoxicity was greatly reduced by such reducible polycations. Moreover, the transfection efficiency of Poly $\text{HK}_6\text{H-mPEG}$  was comparable to that of PEI-mPEG, but its toxicity was much lower than that of PEI-mPEG.



**Figure 2.** Schematic of the PEGylated reductive polypeptide vector used for gene delivery. Adapted with permission from the authors of [62].

In addition, dendrigraft poly-l-lysine (DGL) polymers that are synthesized by grafting l-lysine oligomers in a repetitive manner have emerged as new kinds of dendritic poly-l-lysine (PLL) derivatives [63,64]. DGL polymers possess a dendrigraft architecture without tertiary amine groups in addition to some unique properties, including narrow polydispersity, a well-defined structure, and a high density of amine groups on the surface, rendering them suitable for gene delivery. Various DGL-based vectors have been constructed by the integration with stimuli-responsiveness or targeting groups to improve gene transfection efficiencies. For example, Liu et al. reported the modification of DGL polymers with phenylboronic acid (PBA) to enhance the endosomal escape ability (Figure 3) [65]. In vitro and in vivo results demonstrated that the PBA modification strategy significantly improved the transfection of siRNA in MDA-MB-231 cells and inhibited the tumor growth by effective

siRNA delivery. In another work reported by Ren et al., cleavable PEG was used to modify DGL polymers, generating the resultant DGL(R)-SS-mPEG, for reducing the cytotoxicity of DGL owing to their high density of positive charges [66]. The resultant cleavable polymer enabled the efficient pDNA loading and is able to respond to tumor-relevant glutathione (GSH) conditions for PEG detachment, which is favorable for subsequent intracellular gene delivery. To further enhance the transfection efficiency, Huang et al. designed a multifunctional DGL-based vector by integrating a PEG and cell-penetrating peptide with DGL [67]. As a result, the nano-sized nanoparticles with the loading of the therapeutic gene remarkably enhanced the blood–brain barrier (BBB)-crossing efficiency, cell internalization, and gene transfection in tumor cells, resulting in a strong apoptosis on the tumor site. Such a multifunctional DGL vector represents a potential efficient gene delivery platform for glioma therapy.

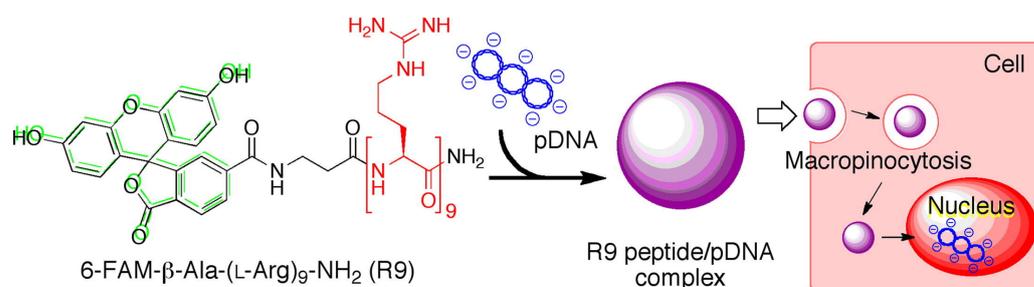


**Figure 3.** Schematic of the synthesis of PBA-DGLs for delivery of siRNA in cancer treatment. (a) Transfection of siRNA by phenylboronic acid (PBA) modified DGL polymers; (b) The structure of PBA-modified DGL polymers Adapted with permission from the authors of [65].

#### 4.1.2. Arginine-Rich Peptides

Arginine (Arg)-rich peptides are another kind of important gene carrier backbone owing to their cationic guanidino groups for interaction with the anionic phosphate groups in DNA or RNA, resulting in the formation of polyion complexes [24]. These kinds of delivery systems have been widely used for the transportation of nucleic acids (e.g., plasmid DNA, antisense DNA, and siRNA) into cells [68,69]. The transfection efficiency and physicochemical properties of Arg-rich peptides are highly dependent on their lengths,

sequences, secondary structures, and additional modifications. In the work reported by Tanaka et al., three kinds of Arg-rich peptides with different numbers of Args, including R9, (RRG)<sub>3</sub>, and (RRU)<sub>3</sub>, were synthesized with the additional modification of a fluorescent molecule (i.e., 6-FAM) for the delivery of pDNA (Figure 4) [70]. As a result, the R9/pDNA complex displayed the most efficient cellular internalization ability and transfection efficiency among the three peptides, since the unique cell-penetrating ability of R9 was remarkably reduced by the replacement of Args with Gly and Aib.



**Figure 4.** Schematic of the synthesis of 6-FAM-labeled R9/pDNA complex for gene delivery. Adapted with permission from the authors of [70].

#### 4.1.3. Poly-L-ornithine (PLO)

PLO has a positive charge under physiological conditions and can bind DNA via electrostatic attraction. Ramsay et al. showed that PLO was stronger than PLL in binding DNA under the same conditions, and the transfection efficiency of PLO was five times that of PLL when transfecting A549 cells. Similar results were obtained when transfecting COS-7 cells [71]. Thomas et al. found in the *in vitro* transfection experiment of B16 melanoma cells that the transfection efficiency was PLO > PLL > Polyarginine. They also studied the transfection efficiency of co-amino acids (co-polymer(Lys:Ala)), and found that PLO >> Lys:Ala(2:1) > Lys:Ala(3:1), while Lys:Ala(1:1) had almost no transfection activity [72]. PLO, when combined with a DMSO, resulted in the shock-mediated efficient transfection of CHO cells. The ability to directly and effectively transfect cells in a growth-arrested state was reported for the first time. This method has also been proven to be effective for other cells including rat embryonic fibroblasts in both stable and transient transfection [73]. The combination of PLL and PLO can form amphiphilic colloidal vesicles, reduce toxicity, and does not rely on receptor-mediated endocytosis. Uchegbu et al. modified PLL and PLO with palmitoyl and polyethylene glycol monomethyl ether (mPEG) to obtain the amphiphilic copolymers PLP and POP [74]. PLP and POP could self-assemble into polymer vesicles in the presence of cholesterol. Their *in vitro* cytotoxicity decreased by 1–2 orders of magnitude, but their hemolysis ability was stronger than that of the PLL and PLO homopolymers. The surface of this type of polymer vesicle was close to neutral and rich in PEG, so it was beneficial to perform transfection *in vivo*. The polymeric vesicle–DNA complexes improved gene transfer to human tumor cell lines in comparison to the parent homopolymers despite the absence of receptor-specific ligands and lysosomotropic agents such as chloroquine. Zhuo et al. synthesized multi-arm star-shaped polyornithine PEI-P(Orn)(n) and grafted polyornithine arms onto branched PEI with a M-w of 600 via ring-opening polymerization [75]. In view of the excellent cell membrane penetration property of being rich in arginine, the amino side groups on polyornithine arms are partially guanidinated and transform the ornithine units to arginine units. The results show that star-shaped poly(ornithine) modified by guanidine salt improved serum compatibility, reduced cytotoxicity, and improved transfection efficiency.

### 4.2. Peptides Act as Functional Moieties

#### 4.2.1. Cell-Targeting Peptides

The positive charge of the cationic carrier is a double-edged sword. Although the interaction of positive and negative charge is beneficial to improve transfection efficiency,

it also leads to the non-specific combination of positively charged complexes with various cells [76]. An ideal gene delivery vector should be able to selectively deliver targeted genes to diseased organs or cells without affecting normal tissues/cells. Otherwise, the therapeutic gene needs large doses of administration to achieve the required efficacy, which will not only cause the waste of active substances, but also increase the risk of side effects. To this end, the main strategy is to attach specific targeting ligands, such as epidermal growth factor, transferrin, folic acid, and, especially, peptides. The RES capture and PEG dilemma of gene delivery carriers are at the appropriate place. The unwanted nonspecific elimination of gene delivery vectors by reticuloendothelial cells, particularly liver sinusoidal wall cells (Kupffer cells and sinusoidal endothelial cells), is one of the most significant barriers to clinical translation, which causes a profound decrease in the delivery efficiency of gene medicines into the target tissues and raises toxicity issues [77]. Surface shielding by PEG onto gene delivery carriers drastically decreases the cellular uptake and endosomal escape, thus resulting in precluded transfection efficiency (PEG dilemma), although PEGylation substantially improved the colloidal stability and stealth in the harsh biological milieu [78]. To overcome this dilemma of PEG shielding on reduced cellular uptake, targeting ligands were strategically placed at the distal end of the PEG segments to promote specific ligand-receptor-mediated uptake [79].

With the rapid development of phage display technology, many peptide fragments corresponding to the functional domain of ligand molecules have been developed [76]. These targeted peptides can be used as ligands to intentionally deliver DNA to specific types of cells [28,80]. The commonly used targeting peptides and their corresponding receptors and sequences are presented in Table 1. Arg-Gly-Asp (RGD) is an integrin-binding peptide, which can bind to many different integrins that play important roles in cell migration, cellular uptake, intercellular interaction, and signal transduction [81]. Therefore, many peptides with an RGD sequence have been developed to target integrin receptors. In addition to this, more and more specific targeting peptides have been found and isolated, such as tumor-homing peptides, vascular-homing peptides, and breast-tissue-homing peptides [82,83]. In order to treat neurodegenerative diseases (e.g., Parkinson's disease or Alzheimer's disease), some peptides that can target neuronal cells have been discovered. For example, neurotensin (NT) is a biologically active single-chain peptide that exists widely in the nervous system of mammals [84]. Arias-Montañó et al. used SPDP to connect NT to PLL and targeted cell lines with a high expression of NT receptors (NTRH), such as N1E-115, HT-29, etc., while in COS-7 cells lacking NTRH, there is no expression at all, which showed its strong targeting selectivity against different cell lines [85].

In spite of this, many cell-targeting peptides have been employed for the construction of peptide-based gene delivery systems with high specificity. For example, Kim et al. synthesized a PEG-g-PLL-based vector conjugated with the artery-wall-binding peptide (AWBP) [86]. Since AWBP provided targeting to the arterial wall cells, AWBP-PEG-PLL was investigated for the gene therapy of cardiovascular diseases. It was shown that the delivery of AWBP-PEG-PLL/pDNA complexes could be mediated by the specific targeting peptide (i.e., AWBP) to the bovine aorta wall cells. Moreover, NGR is another peptide motif obtained via *in vivo* screening, and it has been found *in vivo* that NGR can selectively bind to aminopeptidase N overexpressed on tumor endothelial cells [87]. Cristiano et al. constructed a targeted non-viral vector combining a CNGRC peptide with PEG-PLL, and this vector could specifically target and transfer genes to tumor cells with a positive CD13 expression, thus improving the PEG-PLL-mediated transfection efficiency by 12 times [88]. Hepatocellular carcinoma (HCC) is one of the most common types of malignant tumors worldwide. Zheng et al. fused the HCC-specific peptide SP94 (SFSIIHTPL) to the N-terminus of nine arginine residues via a four-glycine linker, achieving the HCC-specific delivery of siRNA payloads [89]. In addition, AP1 (an IL-4 receptor specific ligand), as a specific tumor tissue and atherosclerotic plaque-homing peptide, can selectively bind to interleukin-4 receptors (IL-4Rs) overexpressed on cells [90]. Park et al. constructed a non-viral gene-delivery system based on the integration of an

elastin-like peptide (EL), Tat, and AP1 for cancer therapy [91]. The resultant ELPs could self-assemble into nanocomplexes that were stable under physiological conditions and, specifically, target to IL4-receptor-expressing tumors. This system was found to deliver plasmids harboring the siRNA to tumor cells and silence the gene of interest. Moreover, Liu et al. constructed a multifunctional peptide TAT-H6-K(C18)-YIGSR containing a targeting YIGSR sequence, which targets the laminin receptor on the surface of tumor cells [92]. It was found that the vector containing the YIGSR segment dramatically improved the gene transfection efficiency via LR-mediated endocytosis. In addition, Gao et al. designed a nanocarrier of GE11-PEG-PEI, which showed significantly more gene copies to EGFR-overexpressing laryngeal cancer cells *in vivo* than that of PEG-PEI alone. It was indicated that GE11-PEG-PEI was a suitable gene delivery vector for treating EGFR-overexpressing laryngeal cancer [93]. In another work, Tang et al. developed a gene delivery vector of MPC/Ad-SS-PEG, which conjugated an MC11 peptide with PEI, PEG, and adamantyl (Ad) groups [94]. The results of gene transfection efficiency and the tumor-targeting experiments show that the MPC/Ad-SS-PEG system may be a safe and efficient non-viral vector for FGFR-mediated targeted gene delivery for cancer gene therapy. Zhang et al. constructed T7-peptide-modified core-shell nanoparticles (T7-PEG-LPC/siRNA NPs) to deliver siRNAs [95]. Both *in vitro* and *in vivo* experiments demonstrated that EGFR siRNA could be specifically transported into breast cancer cells with the assistance of T7-LPC/siEGFR NPs, as a consequence of efficient receptor-mediated endocytosis for the down-regulation of EGFR expression. As a result, such a targeted delivery system could obviously inhibit the growth of breast tumors with little activation of an immune response.

**Table 1.** Some of the commonly identified cell-targeting peptides.

Name	Receptor	Sequence
RGD	Integrin receptors	RGD-containing peptides
REDV	Integrin receptors	REDV
YIGSR	Laminin receptor	YIGSR
GE11	Epidermal growth factor receptor (EGFR)	YHWYGYTPQNVIGRC
GE7	Epidermal growth factor receptor (EGFR)	YHWYGYTPQNVIGG)2-KGRC
MLPLAT	Fibroblast growth factor receptor (FGFR)	MLPLAT
MC11	Fibroblast growth factor receptor (FGFR)	MLPLATGGGC
T7	Transferrin receptor	HAIYPRH
B6	Transferrin receptor	GHKAKGPRK
B18	Transferrin receptor	SPRPRHTLRSL
Angiopep-2	Low-density lipoprotein-receptor-related protein (LRP-1)	TFFYGGSRGKRNNFKTEEY
NGR	CD13 protein	NGR
SP94	Human hepatocellular carcinoma (HCC)	SFSIIHTPILPL
CAGW	Endothelial cells (Ecs)	CAGW
SP peptide	Neurokinin-1 (NK-1) receptor	RPKPQQFFGLM
RP	Neuropilin-1 (NRP-1) receptor	RPARPAR
VHPK	Vascular cell adhesion molecule 1 (VCAM1)	VHPKQHR
GRP	Gastrin releasing receptor	CGGNHWAVGHLM
IF7	Annexin-1 specific	IFLLWQR
EphA	Pancreatic cells	CHVLWSTRC

#### 4.2.2. Cell-Penetrating Peptides

Cell-penetrating peptides (CPPs) are a class of short amino acid sequences that are able to penetrate the cell membrane with a high efficiency [96]. Owing to this distinct property, CPPs have been widely used for the quick delivery of hydrophilic proteins, peptides, nucleic acids, drugs, and other foreign substances through the cell membrane into the cytoplasm or even the nucleus [97,98]. During this process, the cell membrane remains intact, and there is no significant toxic effect on the host cells. Moreover, this

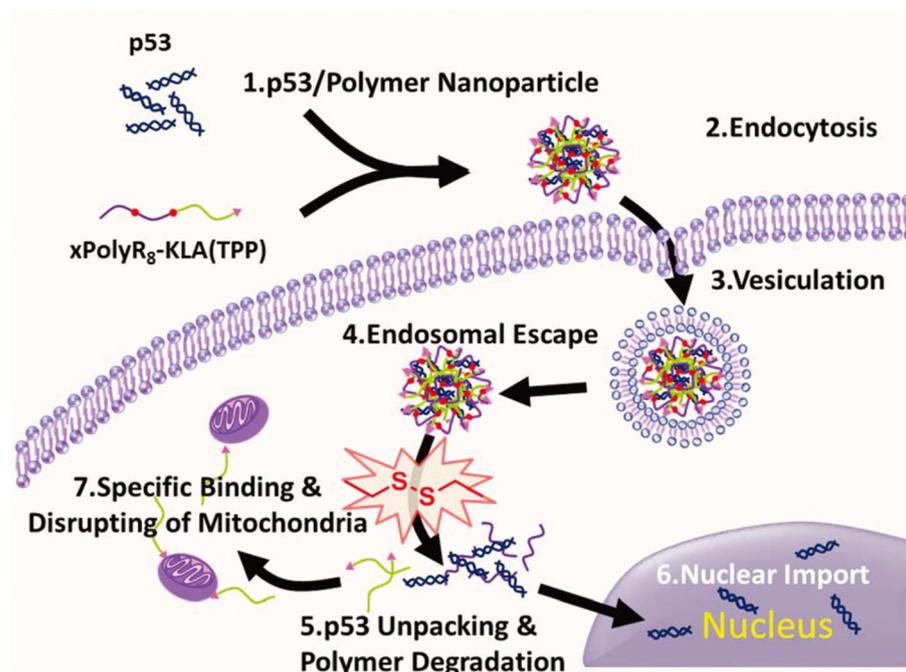
transmembrane effect is not restricted by the cell type. According to the structural characteristics, transmembrane peptides can be divided into two categories: one is a peptide rich in basic arginine represented by TAT (YGRKKRRQRRR), and the other is a peptide with an amphiphilic structure represented by penetratin (RQIKIWFQNRRMKWKK) [99,100]. Since some CPPs are arginine-rich cationic peptides, CPPs can be used either alone for gene transduction or as a transmembrane component in combination with other non-viral vectors. The common CPPs are shown in Table 2.

**Table 2.** Typical cell-penetrating peptides.

CPP Name	Sequence
TAT(48–57)	GRKKRRQRRR
Penetratin	RQIKWFWQNRRMKWKK
MAP	KLALKLALKALKAAALKLA <sup>a</sup>
Transportan/TP10	GWTLNSAGYLLGKINLKALAALAKKIL <sup>a</sup>
VP22	NAKTRRHERRRKLAIER
Polyarginine	Rn <sup>a</sup> n = 8, 9
MPG	GALFLGFLGAAGSTMGA <sup>b</sup>
Pep-1	KETWWETWWTEWSQPKKKRKV <sup>b</sup>
pVEC	LLILRRRIRKQAHAAHSK <sup>a</sup>
YTA2	YTAIAWVKA FIRKLRK <sup>a</sup>
YTA4	IAWVKA FIRKLRKGPLG <sup>a</sup>
M918	MVTVLFRRRLRIRACGPPRVRV <sup>a</sup>
CADY	GLWRALWRLRLSLWRLWRA <sup>b</sup>
SAP	(VRLPPP) <sub>3</sub>
PTD-5	RRQRRTSKLMKR
Mgpe-9	CRRLRHRLHHYRRRWHRFRC
SynB	RGGRLSYSRRRFSTSTGR

<sup>a</sup> C-terminal amide. <sup>b</sup> C-terminal cysteamide.

For example, Sugiura et al. performed cell transmembrane experiments with peptides composed of arginine of different lengths, and found that the peptides composed of 8–10 arginines had the best transmembrane effect [101]. Kim et al. used the reducible poly(oligo-d-arginine) (rPOA) as a pulmonary gene delivery system, which was constitutive of the Cys-(D-R9)-Cys repeating unit [102]. rPOA with disulfide bonds was formed via polymerization of the terminal cysteinyl-thiol groups of the short peptide. An in vivo study showed that rPOA exhibited a higher transfection efficiency than poly(ethyleneimine) (PEI) and sustained non-toxicity for 1 week. In the work reported by Zhang et al., a series of bio-reducible polypeptides were synthesized, which comprise CHK6HC and CR8C in various ratios via reducible disulfide bonds [103]. The results suggest that increasing the amount of R8 can promote the transfection efficiency significantly. Specifically, xPolyK6-R82 exhibited the highest transfection efficiency at a *w/w* ratio of 50. They also synthesized a dual-functional polypeptide (xPolyR8-KLA(TPP)) via disulfide crosslinking of CR8C and C-KLA(TPP) [104]. With an improved cell penetrating ability, the resultant xPolyR8-KLA(TPP) enabled the efficient loading and delivering of therapeutic genes (i.e., p53) and, at the same time, could induce tumor cell apoptosis owing to the cytotoxic effect of C-KLA(TPP) (Figure 5).



**Figure 5.** Schematic of the construction of dual-functional polypeptide (xPolyR<sub>8</sub>-KLA(TPP)) via disulfide crosslinking approach for highly efficient p53 delivery. Adapted with permission from the authors of [104].

In addition, Wang et al. designed a hybrid PEI600-Tat peptide as a gene carrier by covalently linking low-molecular-weight PEI to a bioactive Tat peptide [105]. It was shown that the PEI600-Tat peptide exhibited good biocompatibility and cell membrane permeability, which had a broad application prospect in gene therapy. Bienert et al. proposed an amphiphilic model peptide (MAP) with the sequence of KLALKLALKALKALKLA, and its uptake and transfection efficiency were demonstrated to be higher than those of TAT alone [106]. Omar et al. developed a TAT-conjugated polyamidoamine (PAMAM) dendrimer for the transdermal delivery of DNA vaccines [107]. The results confirm that the TAT-conjugated PAMAM dendrimer could effectively promote DNA vaccine uptake by cells and had a stronger DNA transdermal delivery capacity than the unmodified PAMAM dendrimer. To facilitate the skin penetration of pDNA deeply into the melanoma tissues, Gao et al. constructed a gold nanoparticle (AuPT) conjugated with CPP(TAT) and cationic PEI [108]. The results show that the AuPT could carry pDNA through the intact stratum corneum and efficiently stimulate the intracellular uptake and nuclear targeting of pDNA in cells. Langel et al. developed a gene delivery vector, which crosslinked TP10 to a pDNA via PNA oligomers and then conjugated with PEI [109]. It was shown that, compared with PEI, the transfection efficiency of the conjugated TP10-PEI was several times higher, and its toxicity was lower.

#### 4.2.3. Endosomal Escape Peptides

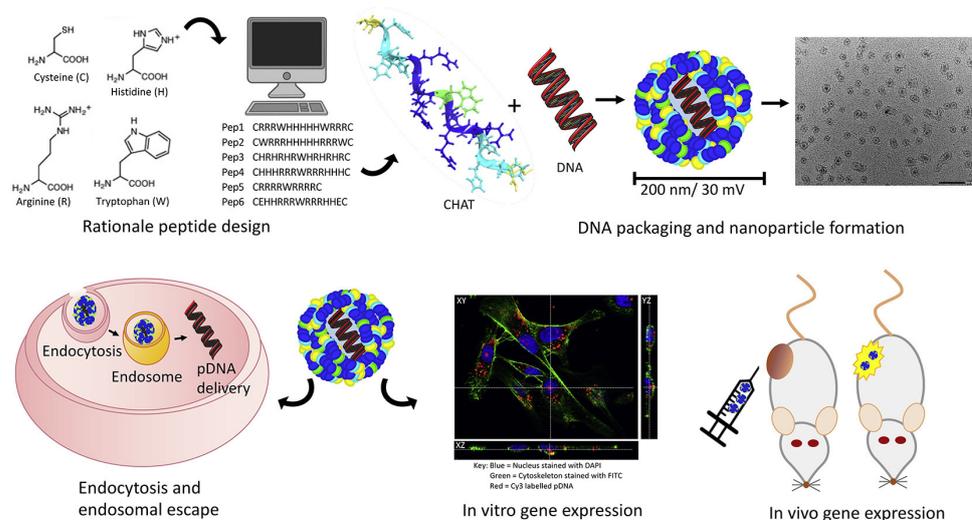
After endocytosis into cells, the vector–DNA complexes are first encapsulated in the endosome and then transported to the lysosome. If the complexes cannot escape from the endosome/lysosome into the cytoplasm as soon as possible, they will face the harsh environment of acidity ( $\text{pH} \approx 4.5$ ) and various hydrolases, which will rapidly degrade and inactivate DNA or the whole vector [110]. Therefore, endo/lysosomal escape has been regarded as one of the most critical bottlenecks of improving the transfection efficiency of non-viral vectors, which may greatly limit the transportation of DNA in gene therapy, resulting in low therapeutic efficiency. Therefore, whether the vector can escape from the endosomal/lysosome quickly has an important impact on the efficiency of gene therapy. Recently, the “proton sponge effect” and endosome disruptors are mainly applied to

solve this challenge [111,112]. Some of the commonly used endosome escape peptides are listed in Table 3. They are originated from various sources, including fusion peptides from the N-terminus of viral fusion proteins (e.g., sHGP, VP1, and INF7), toxins from plants and animals (e.g., melittin), antimicrobial peptides (e.g., magainin2), and synthetic amphiparental peptides (e.g., KALA, GALA, and LK15).

**Table 3.** Typical identified endosomal escape peptides.

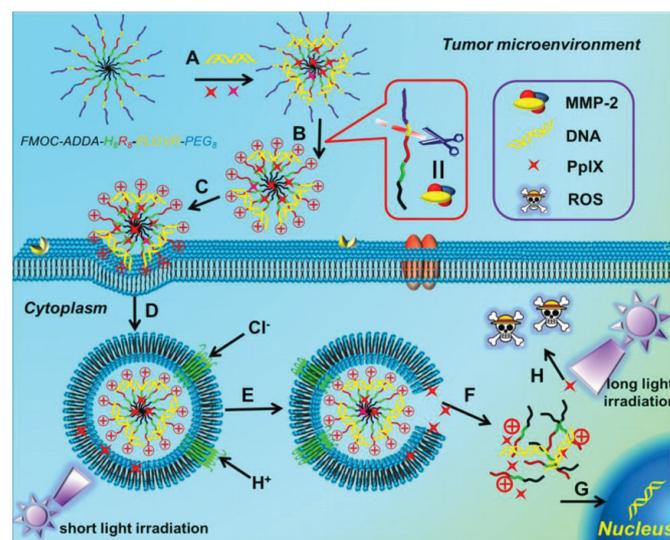
Name	Sequence
KALA	WKAALAKALAKALAKHLAKALAKALKALAA
GALA	WEAALAEALAEALAEHLAEALAEALEALAA
LK15	KLLKLLKLLKLLK
sHGP	RGWEVLKYWWNLLQY
INF7	GLFEAIEGFIENGWEGMIWDYG
VP1	NPVENYIDEVLNEVLVVPNINSSNC
Melittin	GAVLKVLTTGLPALISWIKRKRQQ
Magainin2	GLGKFLHSAKKFGKAFVGEIMNS
H5WYG	GLFHAI AHFIHGGWHGLIHGWYG
HA2	GLFGAIAGFIENGWEGMIDG

Secondary and tertiary amines on gene carriers with a “proton sponge effect” can capture a large number of protons, causing the swelling and rupture of endosomes/lysosomes. Therefore, the gene-carrier complex can quickly escape from the endosome/lysosome after endocytosis into cells. Histidine-rich peptides are a class of endosomal cleavage peptides. Histidine contains an imidazole ring that can be protonated at pH 6; thus, it has an internal endosomal/lysosomal destruction function [113]. Bechinger et al. found that the cationic amphipathic histidine-rich peptide LAH4 (KKALLALALHHLAHLALH-LALALKKA) possesses a high pDNA delivery capacity [114]. Such peptides are thought to efficiently disrupt endosomal membranes. The transfection efficiency of LAH mutants depends on the number and position of histidine residues in the peptide as well as on the pH at which the in-plane to the transmembrane transition occurs. Moreover, they found that binding of the DNA complexes to the plasma membrane is mediated by heparan sulfate proteoglycans and that anionic phospholipids may be involved in the endosomal destabilization process. Moreover, histidine-rich peptides with branched structures, such as HHK4b (83mer) [115], [KHKH2KH2KH2KH2KHZKHK] 4-KK, and HHHHK8b [116], have also been used as pDNA and siRNA delivery vectors. The Tat peptide is a CPP, which can enhance cells’ uptake of drugs and protein. The application of the Tat peptide in DNA delivery is limited by the instability of the peptide-DNA complex and the inability to release DNA in the endosome. In addition, Wang et al. added different amounts of histidine to the TAT transmembrane peptide with cysteine at both ends [117]. They found that when 10 histidines were added into the sequence, its efficiency for transfecting pCAG-luc was the highest, which was 7000 times higher than that of TAT and equivalent to 25 kDa PEI. To greatly enhance the gene delivery efficiency, McCarthy et al. designed a linear peptide, which integrated multiple functional amino acids that play critical roles in the delivery of a functional nucleic acid (Figure 6) [118]. Specifically, arginine (for efficient cellular uptake and nucleic acid loading), tryptophan (to enhance the interaction with hydrophobic cell membranes), histidine (for facilitating endosomal escape), and cysteine (for controlled release and stability) were employed for the synthesis of the liner cell-penetrating peptide termed CHAT, which was subsequently complexed with pDNA to generate the nano-sized nanoparticle. As a result, peptides with all functional amino acids showed successful transfection with efficient reporter gene expression in an in vivo evaluation through intra-tumoral or intravenous delivery.



**Figure 6.** Schematic of the preparation of multifunctional linear peptide with different functional peptide segments for highly efficient gene delivery. Adapted with permission from the authors of [118].

In addition, photochemical internalization (PCI), as a newly emerged technology for release of endocytosed components, has been demonstrated to be an alternative strategy for the improved cytoplasmic delivery in gene delivery [119]. In the work reported by Zhang et al., a pH-sensitive chimeric peptide, that is, Fmoc-12-aminododecanoic acid-H8R8-PLGVR-PEG8, was fabricated for the delivery of therapeutic genes to treat cancer (Figure 7) [120]. With the combination with a photosensitizer (i.e., PpIX), a dual-stage light irradiation strategy could be achieved for the efficient transportation of plasmid DNA. This chimeric peptide could target tumor cells on the basis of matrix metalloproteinase-2 (MMP-2)-triggered targeting. After cellular internalization, satisfactory endosomal escape was achieved, which was attributed to the synergistic effect of the “sponge effect” of H8 and the “PCI effect” of PpIX under short-time light irradiation, which ensured the high expression of therapeutic genes.



**Figure 7.** Schematic diagram of the chimeric peptide/PpIX/p53 system with synergistic effect of the “sponge effect” of H8 and “PCI effect” for endosomal escape. (A) Construction of the nanodelivery system; (B) MMP-2 responsive PEG detachment; (C) Cellular endocytosis; (D) Formation and acidification of endosomes; (E) Endosomal escape; (F) Intracellular diffusion; (G) Nuclear translocation and gene expression; (H) Photodynamic therapy. Adapted with permission from the authors of [120].

Endosome disruptors are generally amphoteric peptide molecules that are rich in acidic amino acids (glutamic acid and aspartic acid). In the acidic environment of the endosome, they can promote the formation of amphoteric helical structures and fuse with the endosome membrane, thereby destroying the endosome and assisting with the escape of the complexes. Ogris et al. covalently linked PEI to either the C-terminus (C-mel-PEI) or the N-terminus of melittin (N-mel-PEI). Compared with PEI, the transfection efficiency of C-mel-PEI resulted in a 160-fold increase [121]. The site of melittin linkage was shown to strongly influence the membrane-destabilizing activities of both conjugates and polyplexes. The study speculated that the high potency of C-mel-PEI to destabilize membranes at a neutral pH may be due to a reported destabilization mechanism proceeding through the membrane insertion of the peptide, while N-mel-PEI is supposed to induce lysis via insertion-independent pore formation according to the toroidal pore model. In order to achieve gene therapy for central nervous system diseases, Pun et al. designed a copolymer (pHgMelbHK10) for the transfection of cells in the brain, which incorporated the membrane-lytic peptide melittin [122]. Its N-terminus is predominantly hydrophobic, while the C-terminus is hydrophilic. Melittin is relatively water-soluble but adopts an  $\alpha$ -helical conformation when in contact with membranes. Its cytolytic activity is based on its ability to insert into the lipid membrane and induce pore formation. The results show that the melittin-modified copolymers possessed more compact morphologies, increased the DNA binding ability, and transfected more efficiently in both neuron-like PC-12 and HeLa cells than the melittin-free copolymer, and melittin-related toxicity was observed. The *in vivo* results further demonstrate that an increase in the luciferase activity by about 35-fold could be produced by the application of melittin-containing polyplexes in the brain than the melittin-free polyplexes. Zimmer et al. reported that the GALA repeat and KALA repeat can effectively improve the transfection efficiency of non-viral vectors [123]. Specifically, a KALA peptide is an amphiphilic peptide that can form an  $\alpha$ -helix structure at a physiological pH [124]. To this end, Harashima et al. reported that the modification of a KALA peptide on a pDNA-encapsulating liposomal membrane could greatly facilitate the transgene expression and immune activation in bone-marrow-derived dendritic cells (BMDCs), rendering the KALA-modified lipid nanoparticle a potential DNA vaccine for efficient anti-tumor immunotherapy [125]. To further explore the minimum unit of the KALA peptide and the importance of its secondary structure for these activities, they further designed and evaluated pDNA-encapsulating multi-functional envelop-type nano devices (MENDs) modified with four short KALAs [124]. It was found that a KALA peptide must form an  $\alpha$ -helical structure to induce cellular uptake in BMDCs, and the KALA3 (WEAKLAKALAKALA) was the shortest segment that could form the  $\alpha$ -helical structure, as well as elicit transgene expression and immune activation in BMDCs.

#### 4.2.4. Nuclear-Localizing Peptides

To play a therapeutic role, DNA must enter the nucleus for the efficient transcription and translation of the target protein. Increasing the amount of DNA entering the nucleus can increase the expression level of the target gene. The nuclear pore complex (NPC) structure of eukaryotic cells allows for the free passage of substances with a diameter of less than 9 nm (equivalent to 40–60 ku of protein or less than 1 kb of DNA) [126]. Substances with a diameter of less than 25 nm can be transported into the nucleus through a passive transport process [54]. However, substances with a larger size require the assistance of transport molecules that can be recognized by cytoplasmic transport receptors, which are called nuclear localization signals (NLSs) [127,128]. The sequences of NLS peptides are presented in Table 4.

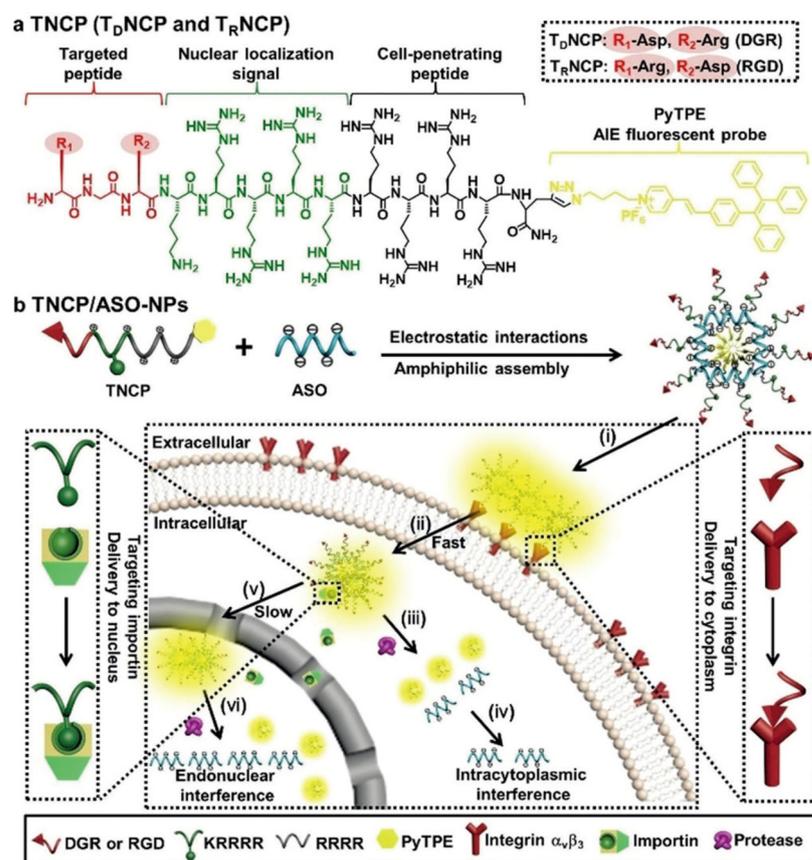
**Table 4.** Typical identified NLS peptides.

NLS	Sequence
SVT40	PKKKRKV
H2B	GKKRSKV
V-Jun	KSRKRKL
Dorsal	RRKRQR
Nucleop lasmin	KRPAATKKAGQAKKKKLDK
NIN2	RKKRKTEEESLKD KAKKSK
SWI5	KKYENVVIKRSPPKRGPRK
RB	RKKKPK-12X-KKSK
PTHrP	YLTQEINKVETYKEQPLKTPGRP
M9	NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY
Pho4	SANKVTKNKSNSPYLNKRKKGKPGPDS
RpL23a	VKSHKKKKIRTSPFTIPKTLTLRRQPKYPRKSAPRRNKLDHY
RpL25	MAPSAKATAAKKAVVKG TNGKKALKVRTSATFRLPKTKLAR

Nuclear-targeting peptides can efficiently transport complexes in the cytoplasm into the nucleus for gene expression. Generally, NLS is a short sequence with monopartite or bipartite clusters of basic amino acids [129]. The import of large molecules to the nucleus mediated by NLS peptides is a two-step program related to the combination with importins and translocation through the NPC, subsequently. The most widely used NLS peptide is derived from the SV40 large T antigen with the minimal sequence PKKKRKV [130]. The reported binding methods include the covalent binding of NLS to DNA, the binding of DNA-PNA to NLS, and the electrostatic binding of NLS to DNA. NLS peptides can be covalently combined with DNA via chemical or photoactive groups, but this nonspecific combination may occupy the interest sites of genes and cause the prohibition of gene expression [131]. NLS sequences can also be ligated to specific sites in DNA via a PNA clamp, and the content of NLS peptides added per pDNA is the key to the success of nuclear entry [132]. Compared with the above two methods, the electrostatic binding method is much simpler in operation and has a higher success rate. Furthermore, this binding mode is reversible and does not affect the expression of pDNA. Since NLSs are usually rich in lysine and arginine, most NLSs are positively charged and can easily bind to DNA and protein via electrostatic interaction, which increases the nuclear input and enhances gene expression. However, electrostatic interaction is a physical method, and there is a possibility of DNA dissociation before the complex reaches the NPC. To this end, a crosslinking approach was reported by Chu et al. to crosslink PEI600-CyD (PC) with low-molecular-weight PEI (600 Da) by  $\beta$ -cyclodextrin ( $\beta$ -CyD), which were subsequently modified with NLS [133]. It was found that the introduction of an NLS peptide greatly increased the expression of transfected DNA in dividing and non-dividing cells. Zhuo et al. designed a series of gene delivery vectors containing the NLS (PKKKRKV) peptide to promote nuclear transport, octamylarginine (R8) to enhance plasmid encapsulation and cellular uptake, and stearic acid to regulate cell membrane permeability [134]. As a result, the prepared vector did not show significant cytotoxicity, while the maximum transfection activity (up to 80% as effective as jetPEI<sup>TM</sup>) could be obtained with the assistance of these NLS-containing vectors. Furthermore, Lam et al. constructed the efficient gene delivery vectors LAH4-L1 that incorporated NLS peptides to promote nuclear importation [135]. Among four kinds of NLSs, SV40-derived NLS and reverse SV40-derived NLS showed the most significant improvement in DNA transfection, which resulted in an increase in gene expression by nearly 10-fold over the non-NLS-modified LAH4-L1.

Recently, Xia et al. reported the design of a versatile gene-delivery strategy for the sequential delivery of an antisense single-stranded DNA oligonucleotide (ASO) in a targeted cell nucleus (Figure 8) [136]. Specifically, a multidomain designer vector, TNCP, was fabricated via the integration of a targeted peptide, NLS peptide, CPP, and an AIE fluorescent probe (PyTPE) to generate the multifunctional vector with distinct properties, including integrin targeting, cell permeability, nuclear import, as well as real-time track-

ing. Compared with the delivery of siRNA that functions in the cytoplasm, the resultant TNCP/ASO complex showed effective gene transcription in a much higher efficient manner, demonstrating the nucleus-targeting delivery. In addition to the nucleocytoplasmic trafficking property, such a design provides a valuable theranostic platform for successful oligonucleotide-based therapies in further clinical applications.



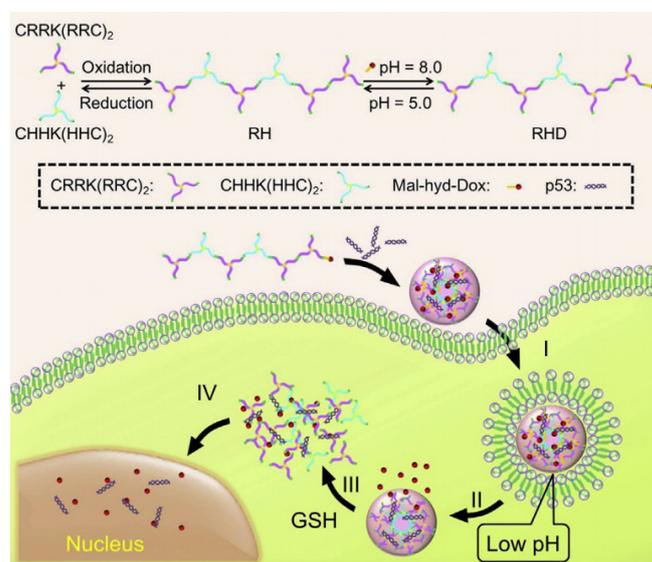
**Figure 8.** The (a) design and molecular structure of TNCP for (b) nuclear delivery and real-time tracking of therapeutic genes. Adapted with permission from the authors of [136].

#### 4.3. Chimeric Peptide for Gene Delivery

Peptides with different functionalities can be integrated into a single system, generating the so-called chimeric peptide [89,137]. These peptides combine the beneficial properties of different kinds of peptides with distinct biological features, which have gained much attention in the delivery of gene therapeutics [138]. To achieve successful gene transportation with a high expression efficiency, the peptide sequence needs to be designed with enough positive charge to achieve the effective compression function of DNA. Therefore, peptides usually need to contain a certain amount of positively charged amino acids (e.g., lysine, arginine, and histidine). As mentioned above, considering the versatility of peptide-based vectors for gene delivery, cell-targeting peptides, CPPs, endosome escape peptides, and NLS peptides can be combined for gene transfer. Generally, there are two ways of covalently binding different peptides: amino acid condensation and disulfide bond crosslinking. For example, cysteine can be introduced at the end of peptide chains with different functions, and then these functional peptides can be polymerized together via oxidation between cysteines to form reducible disulfide bonds. In the work reported by Liang et al., a new chimeric peptide (PL-1, KTLPTPGGGRRRRRRRRR) was developed, in which plectin-1-targeted PDAC (pancreatic ductal adenocarcinoma)-specific and arginine-rich RNA-binding motifs were integrated [139]. The prepared multifunctional PL-1 could condense miRNA (miR-212), resulting in the formation of self-assemble supramolecular nanoparticles, which exhibited good stability in serum condition. More-

over, these nanoparticles showed a much-enhanced delivery efficiency in transporting miR-212 into PDAC cells specifically. Through the co-delivery of the chemotherapeutic drug of doxorubicin, the therapeutic effect in inducing PDAC cell apoptosis and autophagy was dramatically enhanced by PL-1/miR-212 nanoparticles both in vitro and in vivo.

In addition, Zhang et al. combined CPP with NLS and synthesized the peptide sequence of TAT-PKKKRKV for the gene therapy of limb ischemia disease [140]. Both in vivo and in vitro experiments demonstrated that TAT-PKKKRKV was a highly efficient and low-toxic gene delivery vector, which could effectively promote the expression of the protein in the ischemic hind limbs of rats, thereby improving the ability of local angiogenesis. In order to solve the barriers faced by non-viral vectors in gene delivery, such as cell membrane penetration and endosome escape and entry into the nucleus, Liu et al. designed a series of multifunctional cationic peptides for gene delivery by combining CPP(TAT), endosome escape  $\alpha$ -helix peptide (LLKK)<sub>3</sub>, and stearic acid through the sulfhydryl crosslinking of cysteine [141]. Two of them showed a low toxicity and high transfection efficiency. Among them, C18-C(LLKK)<sub>3</sub>-TAT was used to compress and transport PGL-3 plasmid, and the efficiency of luciferase expression was equivalent to that of commercial liposome Lipofectamine 2000 in 293T cells and NIH-3T3 cells. In another work, Zhang et al. fabricated a highly branched and reducible polypeptide (RH), which was synthesized via the oxidation-induced crosslinking of two three-armed peptides (i.e., (CRR)<sub>2</sub>KRRC and (CHH)<sub>2</sub>KHHC), to obtain a dual-responsive gene/drug co-delivery system (Figure 9) [142]. Specifically, the antitumor drug DOX was covalently conjugated with the highly branched polypeptide RH via pH-sensitive hydrazone bonds, resulting in the formation of RHD, which were subsequently complexed with p53. The resultant RHD/p53 nanosystem could efficiently transport DOX and p53 to tumor cells and possess the ability of endosome escape owing to the protonation by (CHH)<sub>2</sub>KHHC. The pH-triggered DOX release and GSH-triggered p53 release showed a much-enhanced effect in tumor cell killing, representing their great potential for synergistic tumor treatment with a much-reduced dose of chemotherapeutic drugs.



**Figure 9.** Transportation of p53 and DOX via dual-responsive gene/drug co-delivery system (RHD/p53) complex for synergistic therapy. Adapted with permission from the authors of [142].

## 5. Conclusions

The goals of constructing a non-viral vector are as follows: high specificity for tumor tissue; high transfection efficiency; and controllable potential safety problems. At present, the transfection efficiency of genes mediated by non-viral vectors compared with viral vectors, and the viral vector still dominates clinical trials. However, the study of their

biological and physicochemical properties will provide valuable references for designing more mature systems in the next step. As non-viral transgenic vectors, peptides have played a variety of functions. Whether they are used as gene delivery vectors separately or as functional elements to be introduced into other non-viral vectors, optimizing the transfection efficiency of peptide-based vectors is still an important direction for future research. With the development of more functional peptides in the research field, it is believed that the research of non-viral vectors will be strongly promoted. In addition, in cancer treatment, the efficient and specific co-delivery of chemotherapeutic drugs and genes with peptide-based vectors to combat drug resistance is a focus of future research.

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