

Core Bottlenecks and Optimization Strategies of Delivery Systems for Cancer Gene Therapy: Intracellular Delivery Barriers, Deficient Targeting, and Insufficient Stability

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Abstract. Cancer gene therapy can intervene in cancer progression at the genetic level, which is an important innovative direction for tumor treatment. Its clinical application is limited by the safety and efficiency of nucleic acid delivery in the body. With the continuous optimization of related technologies and vectors, the academic circle has made key progress in overcoming in vivo delivery barriers and improving material systems. Nevertheless, existing delivery systems still face key challenges such as the difficulty in balancing efficiency and safety, and insufficient adaptability to the complex in vivo environment. The future development of this field will place greater emphasis on the "programmed" delivery concept, and realize precise regulation of vector stealth in circulation, active recognition at tumor sites, and efficient intracellular release through multidisciplinary collaboration of materials science, nanotechnology, synthetic biology and other disciplines. Focusing on the three core bottlenecks of intracellular delivery barriers, deficient targeting, and insufficient stability, this paper systematically reviews the optimization strategies and research advances in pH-responsive materials, membrane fusion peptides, active targeting ligands, cleavable PEGylation, and engineered exosomes, to provide a reference for the rational design and clinical translation of delivery systems for cancer gene therapy.

Keywords: Cancer gene therapy, Delivery systems, Intracellular delivery barriers, Biomedical science, Genetic technology.

1. Introduction

Cancer gene therapy fundamentally corrects the genetic abnormalities of cancer cells by introducing, silencing, or editing genetic materials, bringing revolutionary prospects for the treatment of malignant tumors. From early tumor suppressor gene replacement therapy to current CRISPR/Cas9 gene editing, CAR-T cell immunotherapy, and mRNA vaccines, gene therapy strategies have been evolving, and its application boundary in oncology has been continuously expanding [1,2]. However, despite the great progress in gene manipulation tools themselves, the safe and efficient delivery of therapeutic nucleic acids into target cells remains the main obstacle restricting its clinical translation [3].

The core mission of a delivery system is to overcome multiple biological barriers: protecting nucleic acids from degradation in blood circulation, specifically recognizing tumor tissues, crossing cell membranes, and escaping from endosomes/lysosomes into the cytoplasm or nucleus. Existing delivery vectors can be divided into two main categories: viral vectors (e.g., adeno-associated virus, lentivirus) and non-viral vectors (e.g., lipid nanoparticles, polymeric nanoparticles, exosomes). Viral vectors have high transfection efficiency, but have immunogenicity, carcinogenic risk, and packaging capacity limitations; non-viral vectors have better safety, but often face the dilemma of low delivery efficiency [4]. This trade-off between efficiency and safety essentially reflects the limitation of current delivery systems in coping with the complex in vivo environment.

Starting from the three core bottlenecks of intracellular delivery barriers, deficient targeting, and insufficient stability, this paper systematically reviews the optimization strategies in recent years, and discusses how to promote the clinical translation of cancer gene therapy through the innovation of material design and delivery technology combined with specific data from original studies.

2. Intracellular Delivery Barriers and Optimization Strategies

For gene therapy drugs to exert their therapeutic effects, they must cross the cell membrane and reach specific subcellular locations: mRNA and plasmid DNA need to enter the cytoplasm for translation, while the CRISPR/Cas9 system needs to enter the nucleus to achieve gene editing. However, endosomal/lysosomal entrapment after cellular uptake is widely recognized as the major barrier. Quantitative studies have shown that only approximately 1-2% of nanocarriers internalized via the endocytic pathway can successfully escape from endosomes, while the vast majority are degraded by the acidic environment and hydrolytic enzymes in lysosomes [5]. The root cause of low endosomal escape efficiency lies in the maturation process of the endocytic pathway. Early endosomes (pH ~6.5) are gradually acidified to late endosomes (pH ~5.5), and finally fuse with lysosomes (pH ~4.5), a process accompanied by the activation of multiple hydrolytic enzymes. If nanocarriers fail to escape in time during this process, the nucleic acids they carry will be completely destroyed. Aiming at this bottleneck, researchers have developed a variety of optimization strategies (Table 1).

The design of pH-responsive materials is one of the most classic strategies. Amine-containing cationic polymers such as polyethyleneimine (PEI) promote endosomal rupture via the "proton sponge effect": in an acidic environment, protonation of the amine groups of PEI leads to chloride ion influx and increased osmotic pressure, which eventually ruptures the endosomal membrane. However, PEI has a high charge density, which is prone to induce cytotoxicity in *in vivo* applications [6]. To balance efficiency and safety, researchers have developed ionizable lipids, which are neutral at physiological pH and protonated to cationic in the acidic environment of endosomes, inducing the formation of a hexagonal phase structure in the endosomal membrane and promoting membrane fusion and nucleic acid release. Studies have shown that optimizing the pK_a value of ionizable lipids (ideal range 6.2-6.5) can significantly improve the intracellular delivery efficiency of mRNA, increasing the luciferase expression level by more than two orders of magnitude in animal models [7].

The introduction of membrane fusion peptides is another important direction. Certain viral proteins, such as the influenza virus hemagglutinin HA2, can undergo conformational changes under acidic conditions, insert into the endosomal membrane and induce fusion. Modification of HA2 mimetic peptides on the surface of liposomes can increase the gene silencing efficiency of siRNA from less than 20% to more than 65% [8]. In addition, photochemical internalization technology uses photosensitizers to generate reactive oxygen species under light to destroy the endosomal membrane, enabling spatiotemporally controllable intracellular release in specific scenarios.

Notably, recent studies have begun to focus on the dynamic process and quantitative evaluation of endosomal escape. The fluorescence resonance energy transfer technology was used to monitor the endosomal escape of lipid nanoparticles in real time, and found that the traditionally recognized "proton sponge effect" may not be the main mechanism, while the interaction between ionizable lipids and endogenous anionic lipids in endosomes is the key. This finding provides a new idea for the rational design of high-efficiency escape materials.

3. Deficient Targeting and Optimization Strategies

After systemic administration, gene therapy drugs need to accurately recognize tumor cells while avoiding non-specific distribution in normal tissues. Traditional passive targeting relies on the enhanced permeability and retention (EPR) effect of solid tumors, but the EPR effect has significant species differences and tumor heterogeneity, with limited clinical translation value. Active targeting strategies, which modify specific ligands on the surface of vectors to recognize receptors or antigens overexpressed on tumor cells, are the mainstream direction to improve targeting accuracy.

Ligand selection is the core of active targeting design. The transferrin receptor is overexpressed on the surface of a variety of tumor cells, and modification of its antibody or polypeptide on the surface of liposomes can significantly enhance tumor uptake. Transferrin-modified cyclodextrin nanoparticles loaded with siRNA was designed. The targeted accumulation and gene silencing effect

of such nanomedicines in human tumor tissues has been confirmed for the first time in melanoma patients. Tumor biopsies revealed an siRNA concentration of 1.2 $\mu\text{g/g}$ and an approximately 40% downregulation of target gene mRNA levels.

Table 1. Main delivery optimization strategies based on endosomal escape mechanisms

Optimization Strategy	Mechanism of Action	Key Outcome	Ref.
Cationic polymer (PEI)	Proton sponge effect: protonation of amine groups in acidic environment triggers chloride influx and osmotic swelling, leading to endosomal membrane rupture	Promotes endosomal escape, but high charge density induces cytotoxicity	[6]
Ionizable lipids	Neutral at physiological pH, protonated in acidic endosomes; induces hexagonal phase structure in endosomal membrane to promote membrane fusion	Optimizing pKa to 6.2–6.5 improves mRNA delivery efficiency by >2 orders of magnitude	[7]
Membrane fusion peptide (HA2 mimetic)	Conformational change under acidic conditions, inserts into endosomal membrane and induces fusion	Increases siRNA gene silencing efficiency from <20% to >65%	[8]

Polypeptide ligands are widely used due to their ease of synthesis and screening. The RGD peptide recognizes integrin $\alpha\text{v}\beta3$, which is overexpressed in tumor neovascular endothelial cells. RGD-modified polymeric micelles loaded with anti-angiogenic genes can reduce the tumor microvascular density by 60% in a glioma mouse model and significantly prolong the survival period [9]. In addition, tumor-homing peptides screened by phage display technology, such as LyP-1, can specifically bind to the p32 protein on the surface of tumor lymphatic vessels and tumor cells. When modified on nanocarriers, they can penetrate deep into tumor tissues and deliver drugs to tumor cells far away from blood vessels.

Another breakthrough comes from the application of in vivo CRISPR screening technology. Traditional screening of targeting ligands is mostly performed in vitro, which is difficult to simulate the complex in vivo microenvironment. Through in vivo CRISPR screening, it was found that CDKN1B gene deletion can significantly enhance the persistence and anti-tumor activity of CAR-T cells in the tumor microenvironment in a myeloma model, providing a brand-new target for the targeting optimization of universal CAR-T. This strategy suggests that the optimization of targeting should not be limited to the surface modification of vectors, but can also be extended to the genetic engineering modification of therapeutic cells themselves. Evidently, the convergence of polypeptide ligand screening and functional genomics is reshaping the design logic of targeted delivery. Future dual-optimization systems integrating surface modification with intrinsic cellular regulation will further enhance the precision and depth of cancer therapy.

4. Insufficient Stability and Optimization Strategies

Gene therapy drugs face multiple inactivation risks during in vivo circulation. Naked nucleic acids can be rapidly degraded by ribonucleases in the blood, with a half-life usually less than 15 minutes. Although nanocarriers can provide a physical barrier for nucleic acids, they also face the risk of dissociation and aggregation under dilution, shear force and protein adsorption. Therefore, maintaining the structural integrity of the vector-nucleic acid complex in blood circulation is a prerequisite for effective delivery.

PEGylation is the most widely used stability optimization method. PEG modification can form a hydrophilic barrier on the surface of nanoparticles, reduce serum protein adsorption and reticuloendothelial system clearance, and prolong the circulation half-life. Studies have shown that unmodified cationic liposomes are largely taken up by the liver within 10 minutes after intravenous injection, while the circulation half-life of PEGylated liposomes can be extended to several hours. However, PEGylation has a "PEG dilemma": although it is beneficial to circulation stability, it inhibits cellular uptake and endosomal escape. To solve this contradiction, researchers have

developed cleavable PEG systems, that is, PEG linkers that can be cleaved in the tumor microenvironment or endosomes. PEG linked by pH-sensitive hydrazone bonds is shed in the acidic tumor environment, exposing positive charges to promote cellular uptake; PEG linked by reduction-sensitive disulfide bonds is cleaved in the high glutathione environment in cells, releasing nucleic acid cargo.

Rational design of vector materials also significantly improves stability. It was reported that through a "lock-and-shield" strategy, a delivery platform coated with a hyaluronic acid nanogel and tumor cell membrane hybrid shell (Nanogel@hMVs) was constructed. Among them, the nanogel acts as a "lock" to physically encapsulate nucleic acids, with an encapsulation efficiency of 81.6-89.2%, and achieves tumor-specific release through pH/redox dual-responsive linkers; the hybrid membrane acts as a "shield" to enhance circulation stability, while retaining the homologous targeting ability of tumor cell membranes and the cytoplasmic delivery properties mediated by fusogenic lipids. The vector was stably retained in serum for 30 days, significantly enriched in tumor tissues after systemic administration, achieved a silencing efficiency of more than 70% for the Survivin gene, and reached a tumor inhibition rate of 83.7% in a mouse xenograft model without obvious toxicity.

In addition, engineered exosomes, as endogenous delivery vectors, have excellent stability and low immunogenicity due to their natural membrane structure. By genetically engineering donor cells, targeting ligands can be displayed on the surface of their secreted exosomes or therapeutic nucleic acids can be loaded. Studies have shown that exosomes loaded with the CRISPR/Cas9 system can be detected for gene editing events in tumor tissues after systemic injection, with an editing efficiency of approximately 3.5% and no off-target effects detected [10]. Although the absolute efficiency still needs to be improved, exosome delivery provides a new option for low-immunogenicity, long-circulation gene therapy.

5. Conclusions

The clinical translation of cancer gene therapy is highly dependent on breakthroughs in delivery systems. At present, intracellular delivery barriers, deficient targeting, and insufficient stability constitute the three core bottlenecks restricting delivery efficiency. Aiming at endosomal/lysosomal entrapment, rational design of ionizable lipids and pH-responsive materials has significantly improved the escape efficiency; aiming at deficient targeting, ligand modification and biomimetic membrane coating have achieved tumor-selective accumulation; aiming at insufficient stability, cleavable PEGylation, lyophilization process optimization and engineered exosomes provide multi-dimensional solutions.

Notably, the optimization of the above bottlenecks is not carried out in isolation, but restricts each other and needs to be considered comprehensively. Taking PEGylation as an example, there is an inherent contradiction between stability and cellular internalization, which can only achieve a dynamic balance by introducing an environment-responsive shedding mechanism. The future design of delivery systems will place more emphasis on the "programmed" concept, that is, endowing vectors with corresponding functions according to the biological requirements of different stages in vivo: maintaining "stealth" during circulation, exposing ligands at tumor sites, triggering endocytosis on the cell membrane, and rapid escape in endosomes. The realization of this goal requires the collaborative advancement of materials science, nanotechnology, synthetic biology, artificial intelligence and other disciplines. With the continuous evolution of gene editing tools (such as base editing, prime editing) and the continuous iteration of delivery vectors, cancer gene therapy is gradually moving from concept to reality. It is foreseeable that on the basis of breaking through the core delivery bottlenecks, precise, safe and controllable gene drugs will play an increasingly important role in the individualized treatment of tumors.

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