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Research Progress of Non-viral Vectors in Tumor Gene Therapy

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Abstract:

Gene therapy introduces genetic material cells to enhance or modify gene function and treat various diseases, including malignant tumors. However, due to poor resistance to degradation in vivo and difficulties in penetrating cell membranes, nucleic acid drugs need the help of vectors to enter target cells. Therefore, the development of safe and high-efficiency nucleic acid vectors is the key to gene therapy. Gene delivery vectors are mainly divided into two categories: viral and non-viral. The latter is concerned because of its low immunogenicity and high gene capacity. This article will summarize the research progress of common non-viral gene vectors in tumor gene therapy.

Keywords: Non-viral gene vectors, Lipid nanoparticles, Polymer nanoparticles, Inorganic nanoparticles, Tumor gene therapy

1. Introduction

Gene therapy is a treatment that introduces genetic material into target cells in order to enhance the function of functional genes or modify dysfunctional genes. The occurrence of tumors results from the activation of certain oncogenes, the inactivation of tumor suppressor genes, and alterations in apoptosis-related genes, which lead to dysregulation of cell proliferation, differentiation, and apoptosis. Therefore, gene therapy can be employed to introduce foreign genes into tumor cells or other cells in order to rectify overactivated genes or compensate for defective genes, thus achieving the purpose of tumor treatment. With the continuous development of gene therapy targets, tumor gene therapy has exhibited promising prospects.

2. Overview of Gene Vectors

Despite the progress of research, the delivery of therapeutic genes (such as DNA, mRNA, siRNA, and ASO) into targeted cells still faces many obstacles. Firstly, due to the large number of nucleases in the body, nucleic acid molecules are easily degraded. Secondly, nucleic acid drugs must enter the cytoplasm or nucleus to function, but nucleic acid molecules are usually large, strongly hydrophilic, and contain a lot of negatively charged phosphate, making it difficult for them to penetrate the cell membrane and enter the cell. Therefore, therapeutic gene delivery requires efficient and safe gene carriers. Gene delivery systems can be roughly divided into viral and non-viral vectors. Viral vectors, including lentiviruses, adenoviruses, and adeno-associated viruses, have advantages such as efficient gene transfection and highly specific delivery. However, they also have limitations in the size of nucleic acid molecules, and the preparation process is complex and costly. Moreover, there may be immunogenicity, carcinogenicity, and other safety risks[1]. These drawbacks limit the future development of viral vectors, making non-viral vectors a potential alternative. In recent years, non-viral vectors with flexibility and safety in chemical design have received increasing attention. Non-viral vectors possess high gene capacity, low immunogenicity, safety, stability, and a flexible chemical structure design. They are not limited by the size of target gene molecules and have a wider range of applications.

3. Non-viral Vectors and Their Applications in Tumor Gene Therapy

Common non-viral vectors can be roughly divided into three categories based on their physical and chemical structures: lipid nanoparticles, polymer nanoparticles, and inorganic nanoparticles.

3.1 Lipid nanoparticles (LNPs)

Lipid nanoparticles (LNPs) are widely used as an efficient non-viral nucleic acid carriers. They have stable nanostructures in the internal environment and can fuse with the inner membrane, effectively delivering nucleic acids. LNPs are usually composed of ionizable or cationic lipids, phospholipids, cholesterol, and polyethylene glycol lipids.

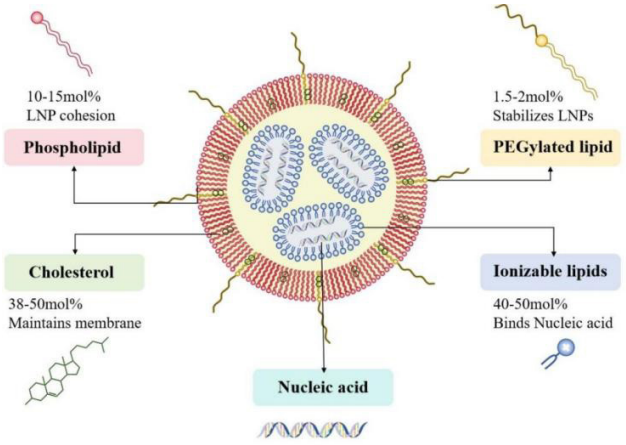

Figure 1 Constitution of LNPs gene vectors

Cationic lipids, due to their positive surface charge, can quickly be neutralized by negatively charged serum proteins and taken up by phagocytic cells, leading to toxicity and reduced efficacy. Ionizable lipids, such as DODAP or DODMA, are not charged under neutral conditions due to deprotonation of tertiary amines, but carry a positive charge under acidic conditions[2]. Therefore, these ionizable lipid nanoparticles can remain neutral in the circulatory system, prolonging their blood circulation time and stability.Helper lipids, such as phospholipids and cholesterol, can form lipid membrane structures and embed nucleic acids in LNPs. Phospholipids, due to their biomimetic properties, contribute to the aggregation and cellular delivery of LNPs. Cholesterol also plays an important role as the maintainer of LNPs membrane, avoiding nucleic acid leakage and improving blood circulation[3]. Polyethylene glycol lipids are synthesized by hydrophilic polyethylene glycol and hydrophobic lipids, which can reduce the non-specific protein adsorption of the carrier, thereby improving the cyclic half-life of LNPs and avoiding clearance by phagocytes.

However, unmodified lipid nanoparticles also have limitations as they accumulate in the liver. Specific ligands and other components can be added to LNPs formulations to increase their accumulation at targeted sites. Patel et al. developed amino acid-modified biodegradable lipids for delivery of siRNA to cancer cells. siRNA-LNPs show very low cytotoxicity and high gene delivery efficiency, and can effectively silence IKKα and IKBKE in prostate and pancreatic cancer[4]. These results suggest that amino acid modification can be used as an effective strategy for improving gene delivery liposomes.

3.2 Polymer nanoparticles

The polymers used to synthesize gene vectors are mostly cationic polymers with high positive charge density, which can bind and compress negatively charged nucleic acid molecules to form smaller polyelectrolyte complexes to protect nucleic acids; At the same time, the excess positive charge on the surface of the complex can make it easier to access the negatively charged cell membrane and thus enter the cell. Common polymers used as gene carriers include polyethyleneimine (PEI), polylysine (PLL), polyamide amide (PAMAM), chitosan (CS), etc.[5].

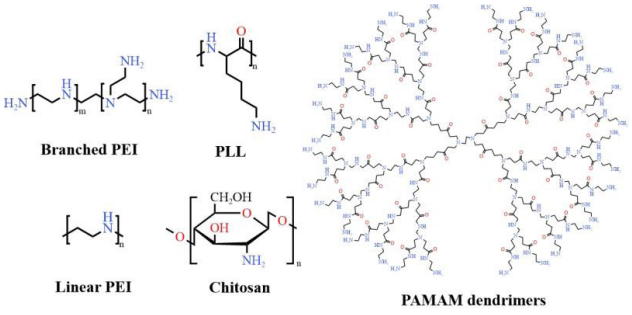

Fig.2 Chemical structure of polymers commonly used as gene carriers

3.2.1 PEI

PEI vectors exhibit excellent transfection efficiency in gene delivery, which is attributed to a so-called “proton sponge effect”, which means the aprotic amines of PEI can absorb H^+ pumped into the lysosome, resulting in an increased influx of Cl^- and H_2O, and then causing osmotic swelling and rupture of the endosome , thereby protecting the captured nucleic acid from degradation[6]. High cation charge density is beneficial for nucleic acid condensation, cellular uptake, endosome escape, and transgenic expression. However, the destruction of endosomes and non-specific binding of anionic molecules can also lead to cytotoxic effects. Low molecular weight PEI has lower cytotoxicity but cannot effectively bind to nucleic acids, while high molecular weight PEI significantly increases cytotoxicity.To reduce cell toxicity, neutral hydrophilic polymer polyethylene glycol (PEG) can be introduced to mask the high cationic charge to reduce the non-specific interaction between the complex and blood components and prolong the blood circulation time of the complex. Another way to reduce the cytotoxicity of PEI cells is to compensate for cell transfection by cross-linking PEI with lower molecular weight. Diaz et al. synthesized cationic graft copolymers containing hydrophobic frameworks and linear PEI (LPEI) through ring-opening polymerization and click chemical reaction. Higher hydrophobicity and higher graft density can strengthen the interaction between DNA and the copolymers and improve transfection efficiency[7].

3.2.2 PLL

PLL is a polymer composed of L-lysine monomers, which

has the advantages of biocompatibility and biodegradability. Research has found that the efficiency of DNA condensation and transfection increases with the increase of PLL molecular weight, but the cytotoxicity also increases. Therefore, when PLL is used as a gene carrier, it often needs to be modified. Urello et al. modified PLL with morpholine to enhance lysosomal escape of DNA and trigger degradation of the carrier material by introducing pH sensitive groups. They also enhanced the stability of nanoparticles by installing hydrophobic groups, achieving low toxicity, high buffering ability, high stability, and high transfection efficiency of the carrier material[8].

3.2.3 PAMAM dendrimers

Dendrimers are macromolecules formed by repeating and linearly connecting oligomers through branching units. With the increase of aggregation algebra, the degree of branching continues to expand, ultimately forming a closed spherical structure. The size of dendrimers can be changed by adjusting their generation, and the molecular surface can easily couple with different molecules to achieve functionalization, making them have broad application prospects in gene delivery.

PAMAM is a typical dendrimers with a large number of positively charged amino groups that can bind to nucleic acids through electrostatic interactions. Research has found that higher dendritic molecular generations (such as G7 and G9) have higher transfection efficiency, but also have higher toxicity. In order to balance transfection efficiency and cytotoxicity, lower generations (<G4) are often chosen for gene transfer, and the transfection efficiency of PAMAM is improved by chemical modification[9].For polymer nanoparticles, targeted ligands (such as folate, RGD peptides, etc.) can be attached to the surface of the nanoparticles to enhance cell uptake specificity through specific recognition between the targeted ligands and receptors on the surface of the target cells. Xu et al. found that the G4 PAMAM dendritic polymer modified with folic acid (FA) as a siRNA carrier exhibited high tumor cells uptake and long retention time in mouse head and neck cancer models[10]. In another study, Lu et al. used RGDyC peptide, which has tumor targeting function, to modify polyethylene glycol functionalized PAMAM. The RGDyC peptide ring can specifically bind to integrins that are highly expressed in glioma cells αVβ3. Experiments have shown that this carrier has the ability to target tumors[11].

3.2.4 CS

Chitosan is a natural linear alkaline polysaccharide, usually obtained by deacetylation of chitin. The primary amine in the chitosan framework protonates at slightly acidic pH, generating a positive charge that can bind to nucleic acids through electrostatic forces. Research has found that chitosan with high degree of deacetylation can generate high-density positive charges, bind more tightly to nucleic acids, and also improve the ability to interact with the surface of cell membranes, thus facilitating cellular uptake; On the other hand, chitosan with lower molecular weight exhibits a higher transfection rate than high molecular weight chitosan[12]. Therefore, chitosan with higher deacetylation degree and shorter molecular chain has more potential to be used as gene carrier. In order to improve the efficiency of gene transfection, Li et al. created a pH sensitive chitosan guanidine biomolecule (CS-DM Agm), which condensed siRNA into nanocomposites. The surface charge of CS-DM-Agm can shift from negative to positive as the pH value decreases, thereby maintaining stability in the blood and higher transfection efficiency in weakly acidic tumor microenvironment[13].

3.3 Inorganic nanoparticles

Inorganic nanoparticles, which usually include gold nanoparticles, silica nanoparticles and iron oxide nanoparticles, have been widely studied for nucleic acid delivery and molecular imaging. They can be designed to specific sizes, structures and geometries and thus have unique advantages.

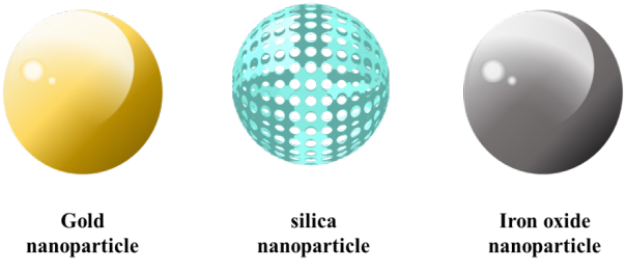

Fig.3 Inorganic nanoparticles commonly used as gene carriers

3.3.1 Gold nanoparticles

Gold nanoparticles are easy to synthesize and surface functionalize. Anionic nucleic acids can covalently connect to the core of gold nanoparticles through thiol groups; Nucleic acid can also be adsorbed by encapsulation with a cationic layer. Sardo et al. coated amphiphilic hydroxyethyl acetamide copolymer (PHEA-PEG-EDA-LA) onto gold nanoparticles to prepare nanogold polymer hybrids (AuNCs), which can protonate and fully chelate with nucleic acids[14]. By adding targeted ligands, gold nanoparticles can also be encapsulated, enabling specific binding between nanoparticles and cell surface receptors. Peng et al. coated AuNPs with antimicrobial peptides (PEP) to assist the delivery of AuNPs to bone marroe-de-

rived mesenchymal stem cells (MSCs)[15].

3.3.2 Silica nanoparticles

Silica nanoparticles have been used as gene carriers due to their excellent biocompatibility and structural adjustability. Typically, nucleic acid molecules are loaded into silica nanoparticles by weakly acting non-covalent structures. Mesoporous silica nanoparticles (MNS) are the most commonly used type. The size of mesopore and the degree of surface functionalization determined the particle loading and nucleic acid release rate. Small pores can limit the release rate of small molecular nucleic acids, while larger pores can provide a faster release rate. For example, silica nanoparticles with small pores (2.5-5 nm) are suitable for delivering small siRNA, while those with large pores (more than 15 nm) are suitable for carrying large DNA. The anionic surface of silica nanoparticles can be coated as a cationic surface to enhance their adsorption and delivery of nucleic acids. Wang et al. used polyethyleneimine (PEI) modified SiNPs to carry MDR1 siRNA for the treatment of oral cancer. The experiment showed that SiNPs PEI MDR1 siRNA was effectively transfected into KBV cells in vitro and induced cell apoptosis[16].

3.3.3 Magnetic nanoparticles

Magnetic nanoparticles, such as iron oxide, show unique advantages in therapeutic vectors and magnetic resonance imaging (MRI). Among them, superparamagnetic iron oxide nanoparticles (SPIONs) are a highly anticipated delivery carrier. Superparamagnetic iron oxide nanoparticles (SPIONs) are a highly anticipated delivery carrier. Applying an external magnetic field can target SPIONs to specific positions, and after removing the magnetic field, magnetic interactions between particles will stop, thereby preventing the accumulation of SPIONs[17]. Modifying nanoparticles can significantly improve transfection efficiency. Veiseh et al. used polyarginine (pArg) to modify superparamagnetic iron oxide nanoparticles with a polyethylene glycol (PEG) coating and further covalently bound with siRNA to transfect rat C6 glioma cells. The results showed that the nanocarrier coated with pArg significantly enhanced the efficacy of delivering siRNA[18]. In addition, SPIONs have high sensitivity in MRI, which can alter relaxation time and generate significant contrast to improve visualization of tumor areas[19]. Therefore, delivery systems based on SPIONs can effectively achieve integrated diagnosis and treatment of tumors.

4. Conclusion

Further research on non-viral gene vectors will help address issues such as immunogenicity and safety of viral vectors. With the rapid development of material chemistry and tumor pathogenesis research, the types of non-viral gene vectors for tumor therapy are becoming increasingly diverse, as well as their functions. At present, many gene therapy targets for tumors have been discovered. Due to the wide variety of nucleic acid drugs, different mechanisms of action, and vastly different molecular sizes, it is unrealistic to use only one "universal" carrier to solve all nucleic acid drug delivery problems. For example, small nucleic acid drugs such as miRNA and siRNA can be delivered by direct coupling with the carrier, while larger nucleic acid drugs such as plasmid DNA and mRNA require the compression and encapsulation of carriers. In addition, small nucleic acid molecules require a carrier with a high positive charge density to form stable complexes, while large-sized nucleic acid drugs have lower requirements for carrier charge density. Due to the significant differences in carrier requirements at different stages of the delivery process, intelligent and environmentally responsive carriers with tumor cell targeting, pH sensitivity, and multiple functions will have greater advantages.

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