Progress In the Application of Nano-Delivery Carriers in Tumor Gene Therapy

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Abstract. Cancer is a serious public health concern, with its prevalence increasing annually. Compared to the traditional anti-tumor therapies, gene therapies can cure cancer by correcting gene defects and completing cascade amplification in a single transfection. However, due to the complex *in vivo* environment, exposed nucleic acid drugs are easily dissolved and inactive, making *in vivo* application difficult. Consequently, diverse drug delivery vehicles have been established, which increasingly play critical roles in refining the transfection efficiency and safety of gene therapy. In comparison to early virus vectors, nanoparticles have been noticed and researched widely because of their excellent biocompatibility, safety, and flexible physicochemical properties. In this review, we systematically discuss major gene therapy technologies such as siRNA, miRNA, CRISPR/Cas9, and introduce the progress in the application of major nanocarriers (including liposomes, lipid nanoparticles, polymeric nanoparticles, and inorganic nanoparticles) in gene therapy delivery. Finally, we outline the challenges in this field and discuss their potential applications in cancer therapy.

Keywords: Nano-delivery carriers, Gene therapy, Liposome, Lipid nanoparticles, Polymeric nanoparticles, Inorganic nanoparticles.

1. Introduction

Cancer is a globally dominant public health problem, which has led to tremendous trouble to human health. In most countries, cancer is suggested as a leading cause of death among people aged under 70, while this point of view has been demonstrated by the report from the World Health Organization in 2020. Based on the GLOBOCAN database, the number of cancer deaths worldwide is close to 10 million, and cancer deaths in China account for as much as 30.15%. In consideration of the aging problem, the number of cancer deaths in China and the world will continue to grow, causing a huge public burden [1]. Conventional treatments have inadequate efficacy and serious side effects, heavily reducing the living quality of patients as well as failing to completely cure cancer. Therefore, in order to further improve the efficacy and patient survival rate, seeking more accurate and efficient treatment methods to replace conventional therapies, such as surgery, chemotherapy, and radiotherapy, has become an essential direction in respect of tumor therapy. Cancers refer to malignant tissues formed by abnormal proliferation due to gene mutation (such as EGFR, ALK, etc.) or abnormal chromosomal copies during cell division and differentiation. The pathogenic factors of cancer are diverse, including genetics, physical and chemical stimuli, lifestyle, and dietary habits, but all these factors will finally induce oncogenes and tumor suppressor genes to be expressed in disorder, causing healthy cells to transform into tumor cells. Cancer cells are characterized by unlimited proliferation and abnormal differentiation. In addition, they also have the properties of metastasis, invasion, and immune escape.

Gene therapy treats diseases by fundamentally changing genes through inserting, removing, and correcting [2]. In terms of tumor treatment, nucleic acid drugs, as well as gene editing drugs, have been broadly noticed and researched since they have good biocompatibility, specificity, and the capability to cure tumors at the genetic level. Furthermore, due to the good operability of DNA and RNA, gene therapy can support more personalized treatment plans. However, due to the large molecular weight of nucleic acid drugs, they are hard to directly cross the cell membrane. In addition, nucleases in the body can easily decompose them; thus, it is impractical to deliver exposed nucleic acid drugs in vivo [3, 4]. At the same time, gene therapy also faces challenges such as breaking

through tissue barriers, delivering drugs to tumor tissues, and targeting tumor cells. Therefore, the development of drug carriers with high safety, reliability, and controllability is the key to improving therapeutic effects. Viruses were initially considered as potential carriers because they can transfer genetic materials and express them in targeted cells. However, viral vectors have the risk of integrating, genotoxicity, and immunogenicity, making them hard to use in clinical applications [5].

Nano-delivery carriers are novel tools that send drugs to target tissues by binding and storing them in carriers with specialized nanostructures. Nanocarriers, with their excellent biocompatibility, safety, and adjustable physicochemical properties, are regarded as promising and ideal carriers for gene therapy drugs. In the past few years, researchers have attempted numerous directions and achieved significant successes in the utilization of nanocarriers for drug delivery. In respect of cancer gene therapy, nano-delivery systems primarily include liposomes, lipid nanoparticles, polymeric nanoparticles, and inorganic nanoparticles. This review will discuss the applications of gene therapeutic drugs and their nano-delivery systems.

2. Gene therapy for tumors

2.1. Oligonucleotide

Antisense oligonucleotides (ASOs) are a class of chemically synthesized single-stranded oligonucleotide molecules, generally composed of 12 to 24 nucleotides. Based on the Watson-Crick principle of base complementary pairing, ASOs bind to the target RNA fragment to form a double helix structure, thereby preventing the expression of the target gene [6]. ASOs can improve stability and decrease decomposition products by certain chemical modifications [7]. In tumor treatment, ASOs act as RNA interference (RNAi) to silence the expression of oncogenes, thereby achieving therapeutic effects [8, 9]. Qian et al. identified an ASO that can effectively reduce the level of H3-3A mRNA and H3.3K27M protein in diffuse midline gliomas (DMGs), successfully inhibiting tumor growth, prompting stem cell differentiation, and improving survival in a mouse model [10].

Small interfering RNAs (siRNA), a kind of non-coding RNA, are 21-25nt double-stranded RNA fragments stemming from long double-stranded RNA molecules (including RNA virus replicons, transposons, or transgene targets) cut by the Dicer enzyme. Then they are transferred to AGO protein to achieve a regulatory effect [11]. In cancer treatment, siRNA, as an RNAi technology, can regulate specific gene expression of cancer cells, thereby inhibiting cancer development. Shubaash et al. screened an anti-KRAS (Kirsten rat sarcoma viral oncogene homologue) siRNA: siKRAS-LNA. siKRAS-LNA combined with gemcitabine treatment can significantly reduce the tumor volume of pancreatic cancer mice [12]. Ming Li et al. combined FA-PFNB-SIRPα with ultrasound therapy to prompt the polarization of M2 tumor-associated macrophages. This process significantly prevents the non-small cell lung cancer from invasion and metastasis and successfully promotes apoptosis progress [13].

MicroRNA (miRNA) is an endogenous gene expression product that is processed by the Dicer enzyme and loaded onto AGO protein. It can effectively regulate gene expression through translation inhibition, chromatin remodeling, or messenger RNA (mRNA) degradation [14, 15]. Recently, the application of miRNA in tumor treatment has achieved great progress. Jianfei et al. designed and synthesized a series of chimeras based on Lin29A miRNA protein hydrolysis targeting to increase the mature miRNA let-7 level, prevent cancer cells from proliferation and migration, and enhance chemotherapy sensitivity of the tumor [16]. Roberto et al. identified a miRNA, miR-182-3P, through high-throughput luciferase screening that can significantly reduce the level of TRF2 protein. This method effectively inhibited tumor development in the triple-negative breast cancer model [17].

Deoxyribozyme (DNAzyme) is a kind of DNA molecule with single-chain structure, which has various catalytic functions such as RNA cleavage, DNA cleavage, and DNA ligase activity. Jiaqi and colleagues established a Ca2+/(aptamer-deoxyribozyme) metal-nucleic acid framework (MNF) system. Then, they use this system to deliver interferon regulatory factor-1(IRF-1), which

significantly increased the amount of reactive oxygen species (ROS) and enhanced nuclear damage in tumor tissues without damaging normal tissues [18].

2.2. Polynucleotide

Vaccines based on in vitro-synthesized mRNA molecules can express specific proteins to regulate biological or immune activity after delivery to cells. In respect of tumor therapy, mRNA vaccines are normally utilized to encode cancer-related viral antigens, tumor antigens (TAA or TSA), and immune regulatory factors [19, 20]. As a tumor treatment strategy, mRNA vaccines have attracted widespread attention due to their good tolerance, easy degradation, difficulty in integrating into the host genome, non-infectiousness, fast production speed and low cost [21]. Ningqiang et al. combined siRab27a (siRNA) with mSting-miR-122 (mRNA) to significantly inhibit tumor growth and reduce the cytotoxicity of mRNA [22]. Yuebao et al. used CD40-LIS10W mRNA to induce the expression of ICD and CD40L in tumor tissues, thereby activating dendritic cells and inducing various immune processes, involving the promotion of effector T cells. As a result of these immune events, the tumor microenvironment and systemic immune responses are reorganized, thereby promoting anti-tumor immunity and tumor killing processes [23].

2.3. Gene-editing nucleic acid drugs

CRISPR is a group of regularly palindromic sequences found in archaea and a few bacteria that have homology with the genes of foreign bacteriophage [24]. After these sequences are transcribed and processed, the CRISPR RNA (crRNA) produced is essential for archaea and bacteria to resist phage infection [25]. Trans-activating crRNA (tracerRNA) is the reverse transcribed product of the DNA sequence upstream of the CRISPR-associated genes, which works with ribonuclease III and protein Cas9 to guide the maturation of crRNA in the type II CRISPR/Cas system [26]. Single guide RNA (sgRNA) is the new RNA chimera obtained by connecting tracrRNA and crRNA as a whole, which retains the double-stranded structure and functions of guiding Cas9 to identify and cut the specific DNA sequence [27, 28]. As an epoch-making gene editing tool, the CRISPR/Cas9 technique has shown great potential in terms of tumor therapy since it is easy to design and has excellent gene editing efficiency. It can cause DNA double bond breaks, enabling cells to insert, replace, and silence target genes through homologous recombination [29]. Currently, applications of the CRISPR/Cas9 technique in tumor treatment are primarily focused on combining it with immunotherapy to improve the therapeutic effect. Lei and coworkers knocked out growth differentiation factor 15 (GDF15) in Hepatocellular carcinoma cells by using CRISPR/Cas9, reversing the tumor immunosuppressive microenvironment and prompting immune cells to kill tumor cells [30].

3. Nanocarrier system

3.1. Liposome

Liposome is a kind of representative nano-delivery system. It is an ultramicrosphere carrier formed by polar molecules and stabilizers. When amphoteric molecules like phospholipids are dispersed in water, their hydrophobic tails will aggregate collectively to form closed vesicles with a distinct bilayer structure, dividing the water into two parts [31, 32]. Liposomes can be loaded with drugs of various polarities [33]. Liposomes have good biocompatibility and can effectively prevent the loaded gene therapy drugs from being degraded by enzymes in vivo, thereby extending the effective period of drugs in blood circulation [34].

Yu et al. introduced a ‘lock’ to liposome by integrating a traceless ROS-cleavable linker, which was named as polymer-locking fusogenic liposome (Plofsme). This vector could effectively cross the blood-brain barrier. Then, it will fuse only after entering glioblastoma (GBM) tissue containing a high ROS amount, thus the siRNA (siMDK) or CRISPR-Cas9 ribonucleoprotein complexes can be delivered into GBM cells. Plofsme can significantly inhibit the MDK expression level of tumor cells, reduce temozolomide resistance, and increase the survival rate of experimental mice by 40% [35].

Ziyu et al. designed RGD-TAT dual peptide modification liposomes that co-loaded with miR-34a and all-trans retinoic acid (ATRA), a regulator of gap junctional intercellular communication (GJIC). Then the carriers were processed into dry powder inhalers (DPIs). Under the concentration of 200 nM of this drug, the survival rate of tumor cells after 96 h was only around 0.35%, indicating that this method has good permeability to tumor tissues, improves the accumulation of drugs in the lung, and improves the transfection efficiency [36].

3.2. Lipid nanoparticles (LNPs)

LNPs are multi-component systems with a bilayer structure and nanoscale size. They are spherical vesicles composed of ionizable lipids or cationic lipid-like compounds, lipid cholesterol, protective agents, polyethylene glycol-lipid conjugates, and genetic drugs or vaccines [37]. The structure of LNPs can prevent internal drug degradation. Furthermore, compared with traditional carriers, LNPs have good biocompatibility, higher delivery efficiency, effective tissue penetration, adjustable physicochemical properties, and low cytotoxicity [38, 39].

Wonbeom and colleagues developed a novel strategy for conjugating antibodies to LNPs without chemical agents: Grab antibody (GrAb) formed by fusion of a targeting antibody with an apolipoprotein A1. Trastuzumab, a kind of antibody against the HER2 receptor, was attached to LNPs by fusing with apolipoprotein A1. The targeted p53 gene-encoding mRNA can enter HER2-positive cancer cells. After 21 days of in vivo treatment, this approach reduced the weight of the tumor by 4.6-fold and increased p53 expression level by 3.9-fold compared to the control group, showing a higher targeted accumulation rate, stronger mRNA delivery effect, and significantly reduced liver and systemic toxicity [40]. Ha Eun et al. hybridized the LNP surface with nanovesicles (NVs) originating from M1-polarized macrophages. Then they encapsulated immunomodulatory factors within the M1-NVs and loaded Bcl2-siRNA into the LNPs. This approach significantly inhibited Bcl2 mRNA level in CT26 cancer cells, induced cancer cell apoptosis, activated intratumoral immune responses, and obviously suppressed the tumor development in experimental model mice [41]. Jing and colleagues prepared lactobionic acid (LA)-modified LNPs and co-delivered camptothecin (CPT) and miR-145. This approach can efficiently penetrate and internalize into liver cancer tissues, enhance the ability of miR-145 to escape from lysosomes, significantly reduce cell viability (55.31%), and increase cell apoptosis rates (33.64%) [42].

3.3. Polymeric nanoparticles

Polymeric nanoparticles are nanocarriers constructed by designing and preparing various high molecular weight polymers, such as polymers, copolymers, and natural polymers. The main polymeric nanoparticles include polyactic acid-based nanoparticles, chitosan-based nanoparticles, etc. [43, 44] Polymeric nanoparticles have received considerable attention in the area of nucleic acid drug delivery since they are easy to be prepared, modified, and functionalized.

Taehyung et al. prepared polymeric siRNA nanoparticles (PRNs) with adjustable size/surface chemical properties, modified with hyaluronic acid and polyglycine, and their size was reduced by about 25 times. By loading PLK1 siRNA, PRNs reduced the tumor tissue weight of an orthotopic breast cancer mouse model by about 80% at 28 days without obvious systemic toxicity, indicating that the carrier has good biocompatibility and targeting to specific organs [45]. Qiuyu et al. developed a chitosan-polypyrrole nanogel (CH-PPy NG) particle and used a membrane to disguise it. Because the membrane was derived from human PC-3 cancer cells, when these biomimetic nanoparticles were used to co-deliver RNAK siRNA and Docetaxel (DTX) for anti-tumor treatment in PC-3 model mice, the consequences showed that the nanoparticles performed favorable biocompatibility, achieved pH-responsive drug release, and precise drug/gene co-delivery. As a result, the system effectively inhibited the cell cycle of prostate cancer cells, silenced RNAK gene expression, and inhibited tumor growth and bone metastasis [46].

3.4. Inorganic nanoparticles

Inorganic nanoparticles are nanosized inorganic particles with good biocompatibility and stability, which are easy to synthesize and modify. Unlike organic nanoparticles, inorganic nanoparticles have a variety of unique properties, including optical and magnetic properties, which enable them to be potentially useful for drug delivery, tissue imaging, and other aspects in cancer therapy. Inorganic nanoparticles mainly include metal materials, semiconductor materials, and inorganic nonmetal materials.

Gold nanoparticles (AuNPs) are a kind of metal inorganic nanoparticles that have been widely studied. AuNPs are highly inert and have unique optical properties and are non-biotoxic. AuNPs can be prepared into various forms to change their optical properties, including gold nanorods, gold nanoshells, and gold nanocages, which show broad utilization prospects in drug delivery [47]. Weinan et al. created a kind of AuNPs carrying miR-21-3p. These carriers can effectively promote IFN-γ-induced ferroptosis of melanoma cells and synergize with miR-21-3P to resist programmed cell death, thereby improving the immunotherapy effect in the tumor mouse model without obvious systemic side effects [48]. Lin et al. proposed a biomimetic system based on cancer cells: photothermal agents and sgRNA were loaded into gold nanorods (GNRs), and then loaded into cancer cell membrane-derived nanovesicles together with Cas9. This strategy, combined with photothermal therapy, increased the early cell apoptosis rate to 34% and the late cell apoptosis/necrosis rate to 41% by editing the survivin gene in cancer cells, showing a significant synergistic anti-tumor effect [49].

Quantum dots (Qds) are inorganic semiconductor nanocrystals characterized by quantum properties and nano size beneath 10nm. Qds have a narrow excitation wavelength range, significant storage drift, great quantum yield, stable and durable fluorescence, good photochemical stability, and long in vivo circulation time, and have a good targeting effect on tumors [50, 51]. Bong and colleagues used cationic glucosamine/PEI-derived graphene quantum dot (PEI-GQD) to non-covalently deliver CRISPR ribonucleic proteins into PC3 prostate cancer cells, successfully returning the abnormally expressed TP53 gene to a relatively normal standard, which induces a 40% apoptosis rate in cancer cells. Moreover, Cas9 can be imaged by GQDs, laying the foundation of cancer therapy that can be traced through image guidance [52]. Peiwen et al. designed complex graphene quantum dots (GQDs) to label co-delivered miRNA with fluorescent signals in vivo. This complex consists of GQDs and fluorescence-labeled miR-193a-3p and is loaded into extracellular vehicles (EVs). When this miRNA binds to the target oncogene, the fluorescent signal will be activated, which can be used to visualize and assess therapeutic effects. This system can reduce cell viability to (18.1±1.7) % in vitro and has tumor-specific toxicity and good biocompatibility in vivo [53].

Mesoporous silica nanoparticles (MSNs) are widely used inorganic non-metallic nanocarriers. MSNs take advantage of improved drug stability, easy surface modification, high bioavailability, and intelligent drug release [54]. Jingming and colleagues developed a PLZ4 ligand-modified pH-sensitive hollow MSNs for targeted delivery of the CRISPR/Cas9-SAM system to bladder tumors. In comparison to LNPs on the market, these nanoparticles showed stronger lysosomal escape ability and cancer cell transfection efficiency. The particles can simultaneously activate the expression of four target genes, prevent the bladder cancer cells from reproducing and migrating, and facilitate cell apoptosis. Meanwhile, they have excellent specificity and biosecurity in vivo [55]. Iris et al. used MSN as a carrier to deliver miR-200c-3p to breast cancer tumor cells. This strategy can effectively enable miR-200c-3p to escape lysosomes and induce cell cycle stopping in the G2 phase in vitro. The proportion of cells ceasing in the G1 phase decreased by 10%, while cells staying in the G2 phase increased by 10%. In a mouse orthotopic tumor model, its effect has the same cell cycle trend, and it could significantly reduce the tumor volume and show good targeting ability and safety [56].

4. Conclusion

In contrast with traditional cancer treatments, gene therapy can target underlying genetic factors that cause cancer, enabling precise, targeted therapy and becoming a crucial component of modern

cancer treatment. The realization of gene therapy largely relies on approaches of delivering the gene editors to target cells. Nano-delivery systems, due to their excellent physicochemical properties, have become important drug delivery tools. While the carriers mentioned in this review, including liposomes, LNPs, polymeric nanoparticles, and inorganic nanoparticles, possess distinct properties, they share several advantages for gene therapy drug delivery, including excellent biocompatibility, safety, adjustable physicochemical properties, ease of surface modification and functionalization, and enhanced tumor tissue permeability. Despite significant research progress, these systems still face numerous challenges in practical application, hindering their clinical application. These difficulties include, but are not limited to, further enhancing carrier delivery efficiency, reducing production costs, minimizing carrier cytotoxicity, and elucidating the carrier's in vivo metabolic pathway and long-term effects. In addition, standardized methods are needed to evaluate the various properties of nanocarriers to achieve scalable production. After overcoming the above problems, the application of nano-delivery vectors and gene therapy drugs will develop in a more intelligent and personalized direction, and treatment plans will be formulated for different cancer types and lesion sites to achieve precise and effective treatment.

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