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Nanocarrier-Mediated Gene Editing for Improved Tumour Therapeutic Efficacy

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Abstract. Conventional gene editing delivery systems are plagued by poor long-term blood circulation stability, weak active tumour-targeting capability, and severe off-target immunogenicity after systemic intravenous administration, greatly restricting the widespread clinical application of precise minimally invasive malignant tumour therapy. This paper reviews considerable recent preclinical literature to systematically summarize the classification, physicochemical properties, and diverse fabrication techniques of mainstream lipid, polymeric, and inorganic nanocarriers. It further elaborates the core working mechanisms of CRISPR/Cas9 gene editing and several types of nanomaterial-assisted delivery platforms, and comprehensively compares their in vivo antitumour performance along with distinct respective merits and unavoidable inherent biological drawbacks. While nanocarriers substantially enhance the systemic in vivo transport and intracellular endosomal escape delivery efficiency of CRISPR gene-editing components, multiple critical translational bottlenecks still persist, including low cargo loading efficiency and inadequate intratumoural enrichment after intravenous injection. This review sorts out such core technical bottlenecks and highlights prospective research directions, such as multifunctional unified carriers and tumour microenvironment-responsive targeted delivery designs, which provides reliable systematic theoretical support for the subsequent clinical translation of nanomaterial-mediated gene editing against various malignancies.

Keywords: Tumour Therapy, Nanocarriers, CRISPR/Cas9, Nanocarrier-Assisted Gene Editing Technology, Targeted Delivery

1. Introduction

Cancer remains one of the leading lethal diseases worldwide. Conventional treatment regimens including chemotherapy, radiotherapy, and viral gene therapy are plagued by inherent drawbacks such as severe systemic toxicity, tumour multidrug resistance, and uncontrollable immune rejection, which greatly restrict the realization of precise tumour intervention. The CRISPR/Cas9 genome editing technique allows precise alterations of oncogenes and tumour-suppressor genes, which offers an innovative approach to reversing malignant development. Despite this fact, its implementation in clinics is significantly hindered by the low in vivo stability of editing elements, inadequate tumour targeting, and low delivery effectiveness of conventional delivery systems [1]. To overcome these problems, many nanocarriers have been used as a means of CRISPR/Cas9 delivery, comprising lipid

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nanoparticles, tunable polymeric nanomaterials, inorganic mesoporous carriers, and bio-inspired nanomaterials. The existing studies have revealed the molecular mechanisms of tumour metabolism reprogramming by explaining how CRISPR-mediated gene knockout decreases chemoresistance as well as confirming the combinative antitumour effect of the use of delivery tools based on nanocarriers with immune checkpoint inhibitors such as programmed death-ligand 1 (PD-L1) blockade [1]. However, the use of single types of nanocarriers has certain shortcomings: lipid systems are limited by their drug loading capacity, polymeric systems cause some mild inflammatory responses, and inorganic particles present the unresolved risks of long-term biosafety.

The concept of intelligent stimuli-responsive nano-therapeutics is proposed wherein various positive aspects of numerous carrier materials are integrated into one comprehensive delivery system and the limitations of single material nano-platforms are addressed [2]. Recently published articles have presented some challenges concerning physiological barriers of the nanocarrier, such as enzymatic breakdown during blood circulation, endosomal entrapment, and ineffective transport to the nucleus. These difficulties have acted as a basis for the improvement of the CRISPR delivery systems [2]. Although there is significant preclinical evidence proving the efficacy of gene editing by means of nano-cargo, the major challenges in its practical implementation are poor accumulation in tumours, differences in physicochemical properties of the product, and insufficient long-term biosafety assessments on mammals. Current scattered studies only discuss one category of nanocarriers separately, lacking a systematic horizontal comparison of lipid, polymeric, and inorganic delivery systems and a complete summary of their matching application scenarios.

This article reviews recent relevant studies to systematically summarize the classification, characteristics, and preparation strategies of mainstream nanocarriers, elaborate the fundamental principles of CRISPR/Cas9 gene editing and nanomaterial-assisted gene editing technologies, and compare their preclinical antitumour performance, delivery mechanisms, as well as major strengths and unresolved technical limitations. The overall objective is to clarify unique strengths of nanomaterial-mediated CRISPR editing compared with traditional therapies, and pinpoint urgent research directions including multifunctional integrated carriers and physiological microenvironment-adaptive delivery designs. This comprehensive sorting further offers theoretical references for the subsequent translational development of nanogene antitumour therapy, and guides standardized quality control for mass production of nanomedicines to guarantee patient safety.

2. Nanocarrier delivery systems

2.1. Core definition of nanocarriers for delivery

Nanocarriers are delivery systems engineered to encapsulate and transport substances with diverse sources and chemical structures, with applications spanning medicine, cosmetics, agriculture, food processing industries, and daily care products. Within clinical medicine, nanocarrier-based platforms serve as drug delivery vehicles for cancer therapy, vaccine administration, clinical diagnosis, as well as the integrated development of therapy and diagnosis (theranostics). A number of traditional medicinal agents are poorly water-soluble, which limits their effectiveness in clinical practice. Nanocarriers are helpful in overcoming this problem since they allow the encapsulation of cargo substances, prevent payloads from degradation, assist the drugs to reach defined organs or tissues in the human body, and improve bioavailability. In the case of cosmetic products, nanocarriers act as carriers for active substances, which allow them to penetrate the skin. Furthermore, nanocarriers enhance the dispersion and stability of the functional substances and decrease the amount of the active components required in the final products [3].

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2.2. Core classification and key properties of delivery nanocarriers

Nanocarriers were classified into four different categories: inorganic, organic, hybrid, and supraparticles, depending on their origin and chemical compositions [3]. The detailed classification is shown in figure 1.

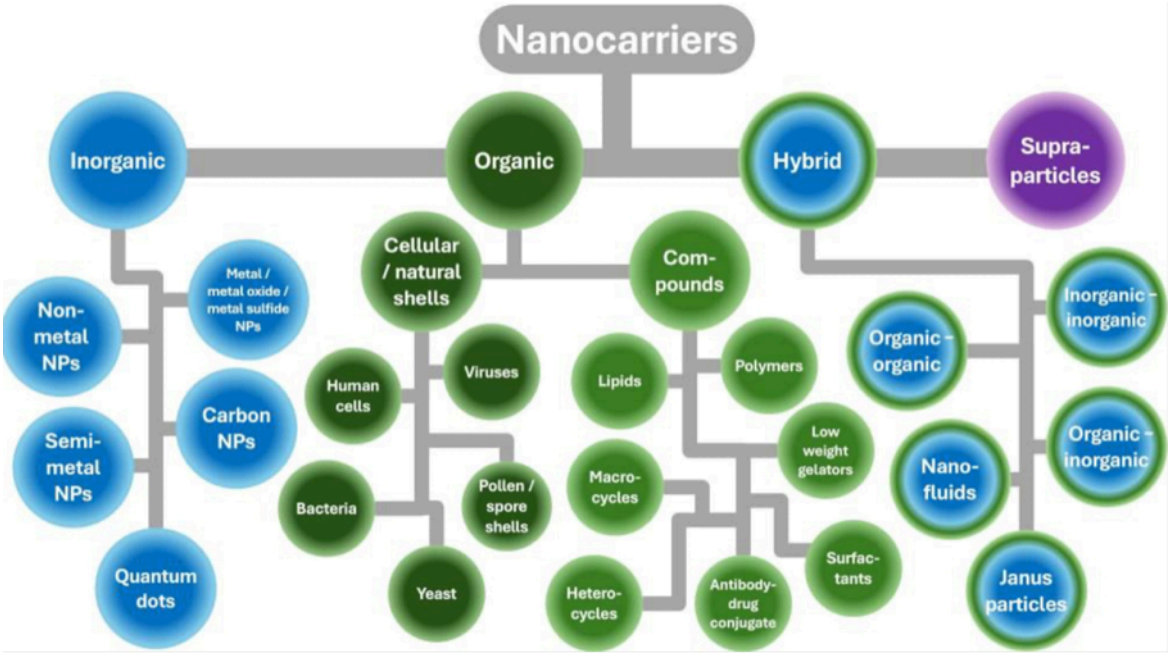

Figure 1. Nanocarrier classification according to their origin and materials [3]

Organic nanocarriers are made using biological templates (human cell-derived vesicles, viruses, bacteria, pollen, spore shells, yeast cells) and synthetic organic materials (polymers, lipids). These carriers have favorable properties, such as good biocompatibility, biodegradability, high loading capacity, amphiphilicity, low systemic toxicity, and facile fabrication. Clinically approved inorganic nanocarriers are mainly represented by iron oxide magnetic nanoparticles (IONPs). They share merits including high cargo loading capacity, acceptable biocompatibility, prolonged blood circulation time, and stimuli-responsive controlled release behavior for encapsulated payloads. Hybrid nanosystems assembled from multiple types of materials can achieve superior comprehensive performance that cannot be obtained from single-component counterparts. Supraparticles are flexible complex aggregates with tunable morphologies and dimensions, self-assembled from one or more types of nano sized building units. They realize unique functions unavailable to isolated nanoparticles, which makes them promising delivery platforms for therapeutic cargos [3].

2.3. Mainstream preparation methods of nanocarrier-based drugs

2.3.1. Emulsification-solvent evaporation

The first and most extensively used method is emulsification-solvent evaporation. The first step consists of emulsifying the polymer solution within the aqueous phase. The second step involves solvent evaporation, which triggers polymer precipitation and ultimately yields nanoparticles [4]. The fabrication procedure of the emulsification-solvent evaporation method is presented in figure 2.

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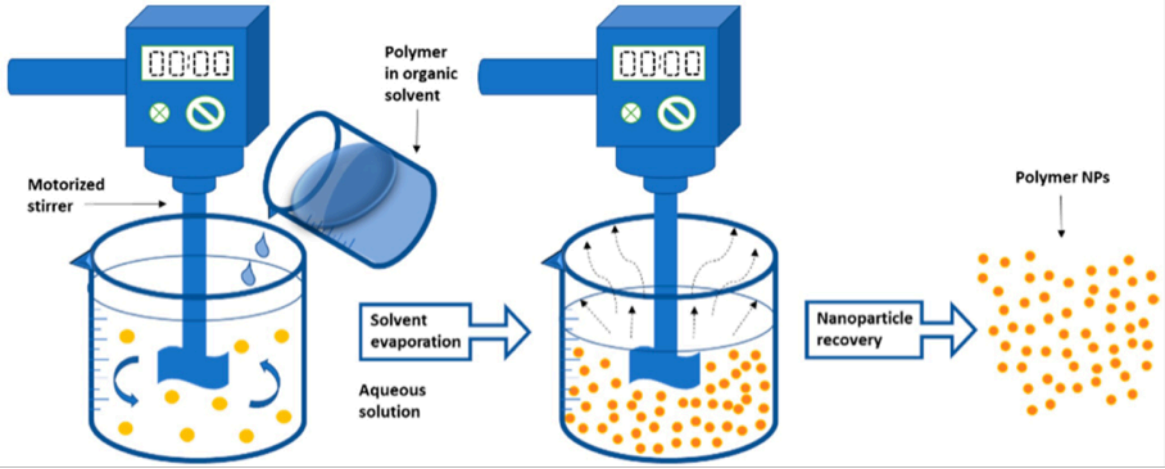

Figure 2. Diagram of the emulsification-solvent evaporation method [4]

2.3.2. Nanoprecipitation

Nanoprecipitation is a straightforward, rapid, reproducible single-step fabrication technique originally developed by Fessi et al. Three major stages are included within this procedure. First, the organic phase is added dropwise into the aqueous phase under moderate stirring to form nanoparticles (NPs). The generated nanoparticles are collected by ultracentrifugation and rinsed with deionized water to remove leftover surfactants. After organic solvent evaporation, nanoparticles solidify and can be further separated through filtration, centrifugation, or freeze drying [4]. The whole preparation process of the Nanoprecipitation method can be seen in figure 3.

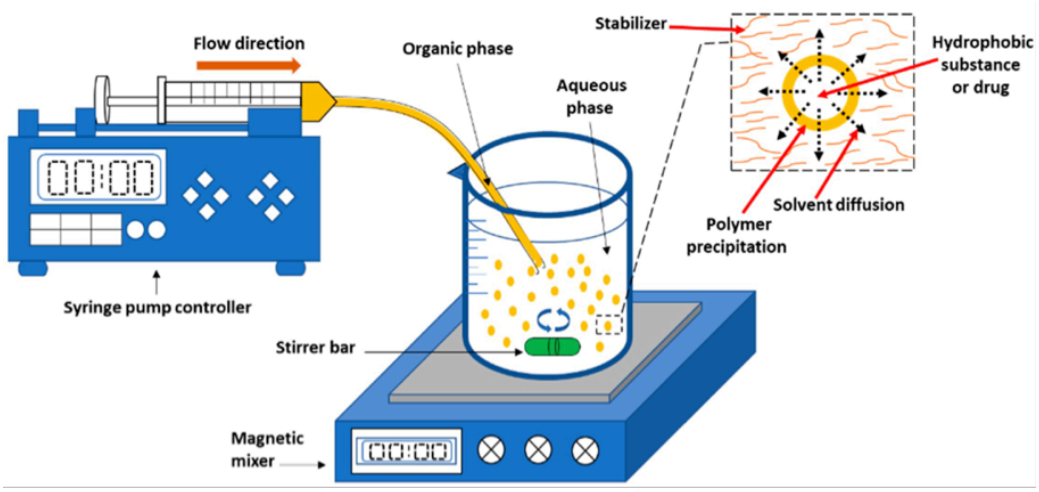

Figure 3. Diagram of the nanoprecipitation method [4]

2.3.3. Sol-Gel

The Sol-Gel method denotes a conventional and scalable technique for producing various nanoparticles. It ensures precise control over the size of the particles and higher purity of the products, uniform appearance, and processing of reactions under low temperature conditions. The procedure begins with the formation of a homogeneous sol formed from precursor materials, and

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then a gel is produced. The steps of solvent removal and drying are performed next in order to obtain finished nanoparticles [5]. The process of sol-gel synthesis is represented in figure 4.

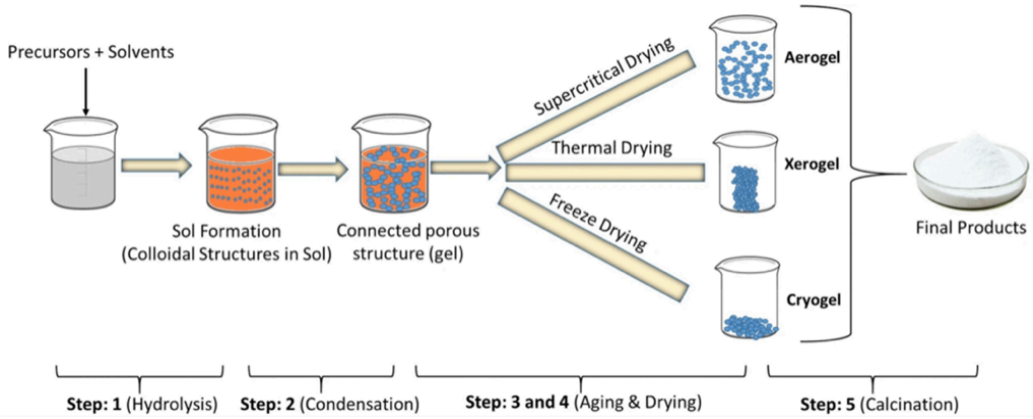

Figure 4. Illustration of how to perform the Sol-Gel technique [6]

3. Core theories of gene editing technology and antitumour mechanisms

3.1. The CRISPR/Cas9 system

The CRISPR-Cas9 system is an efficient and adaptable tool for gene editing that enables accurate genome modification. It utilizes a single guide RNA (sgRNA) to guide the Cas9 endonuclease toward target gene sequences so that DNA can be cleaved with precision, allowing for either a knockout or repair of the gene [1]. Figure 5 shows the two pathways of DNA repair for the CRISPR/Cas9 gene editing process.

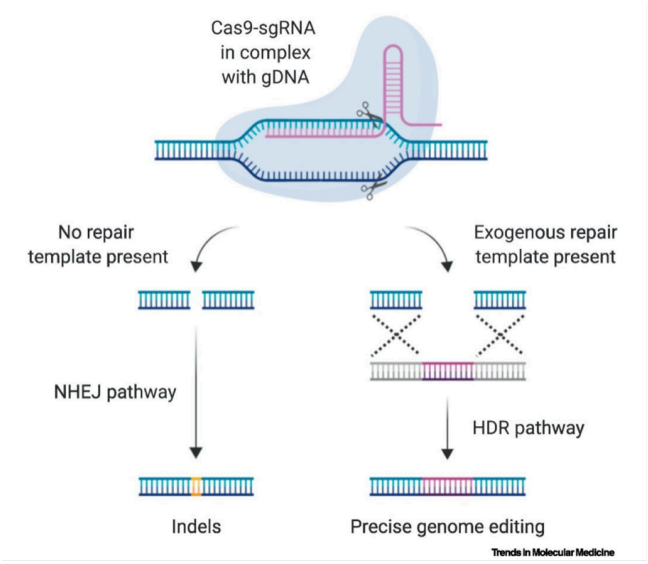

Figure 5. Graphical representation of the CRISPR/Cas9 gene editing method [1]

Numerous varieties of nanomaterials can act as carriers for delivering CRISPR/Cas9, which can be in various forms, such as polymers, polypeptides, lipid nanoparticles, inorganic nanoparticles, dendrimers, and extracellular vesicles. The physic-chemical properties of different classes of nanomaterials are distinctive, which invoke their performance regarding efficient and targeted in vivo delivery [1].

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3.2. Core functional mechanisms of gene editing for antitumour therapy

CRISPR/Cas9 gene editing technology specifically targets oncogenes and tumour suppressor genes and modifies the adjacent tumour microenvironment. Such a gene-editing method prevents tumour growth and enhances the effectiveness of treatment via four different and complementary mechanisms, which will be shown in the following section [7].

3.2.1. Restoration of tumour suppressor genes

Through genetic mutation and epigenetic silencing, tumour cells frequently inactivate tumour suppressor genes (TSGs) that govern cell cycle progression, DNA damage repair, and cellular apoptosis. Advanced CRISPR-based epigenetic editing tools, particularly dead Cas9-Ten-Eleven Translocation 1 (dCas9-TET1)-mediated demethylation, can reverse epigenetic TSG silencing and restore endogenous TSG expression. In glioblastoma animal models, demethylation targeting the Phosphatase and Tensin Homologous deleted on Chromosome 10 (PTEN) tumour suppressor gene successfully rescues the negative regulatory function of the Phosphatidylinositol 3-Kinase/Protein Kinase B (PI3K/Akt) signalling pathway, thereby alleviating tumour burden. Furthermore, base editors enable precise single-nucleotide modification without inducing DNA double-strand breaks (DSBs), significantly minimizing chromosomal rearrangement risks and restoring normal functional protein expression [7].

3.2.2. Oncogene knockout

Oncogenes such as kristen rat sarcoma viral oncogene homolog (KRAS), myelocytomatosis proto-oncogene (MYC), and epidermal growth factor receptor (EGFR) drive persistent cell proliferation, tumour metastasis, and therapeutic resistance following oncogenic activation caused by gain-of-function mutations. CRISPR/Cas9 mediated knockout of oncogenic DNA permanently silences aberrant oncogene activity and blocks downstream oncogenic signalling cascades. Preclinical studies have demonstrated that knockout of the KRAS-G12D (Gly12Asp) mutation in pancreatic cancer (PC) cells suppresses tumour progression. Similarly, simultaneous deletion of MYC and telomerase RNA (TER) in hepatocellular carcinoma (HCC) cells yields synergistic antitumour effects. High-fidelity Cas9 variants and paired nickase systems enable precise targeting of both primary and metastatic oncogenic loci while substantially reducing off-target editing risks [7].

3.2.3. Immunomodulation and immune checkpoint editing

Immune checkpoint molecules, including programmed cell death protein 1 (PD-1), PD-L1, and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), serve as inhibitory proteins that negatively regulate T cell activation and effector functions in the tumour microenvironment. Clinical studies have validated that editing of immune checkpoint genes using CRISPR can successfully restore and improve antitumour T cell immunity. In particular, PD-1 knockout chimeric antigen receptor (CAR) T cells exhibit prolonged persistence in non-small cell lung cancer (NSCLC) patients along with lower rates of side effects as compared to traditional immune checkpoint antibody therapies. Moreover, multiplex CRISPR editing targeting various immune checkpoint loci, combined with genetic modification of cytokine receptors and CARs, can greatly potentiate the infiltration of tumour and cytotoxic killing capacity of engineered T cells. This innovative genome editing

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technology facilitates the development of personalized immunotherapies to eliminate tumour immune evasion [7].

3.2.4. Reversal of chemoresistance

Tumours acquiring multidrug resistance (MDR) often become refractory to conventional chemotherapy. Chemoresistance develops through multiple intrinsic mechanisms, including overexpression of drug efflux pumps such as ATP-binding cassette subfamily B member 1 (ABCB1), upregulation of detoxification enzymes including glutathione S-transferase pi 1 (GSTP1), and enhanced DNA repair capacity mediated by genes such as excision repair cross-complementation group 1 (ERCC1). These resistance-associated genes represent promising targets for CRISPR-mediated knockout to reverse tumour chemoresistance. For example, genetic ablation of ABCB1 impedes drug efflux, thus increasing intracellular paclitaxel accumulation in tumour cells. Likewise, ERCC1 knockout diminishes the capability of DNA repair in tumour cells and makes these cells susceptible to platinum-based chemotherapy again. Overall, editing of certain crucial resistance genes using CRISPR/Cas9 technique is a great strategy to overcome MDR and enhance the treatment efficiency for patients suffering from chemotherapy resistant malignancies [7].

3.3. Core barriers to in vivo application of gene editing-target points addressed by nanocarriers

Three major challenges hamper the employment of CRISPR/Cas9 gene editing technology in clinical practice. First, systemic delivery meets a lot of biological obstacles on both systemic and intracellular levels. Specifically, these barriers include rapid blood clearance, enzymatic degradation of CRISPR components, insufficient cellular internalization, and poor endosomal escape, which collectively lead to low editing efficiency in vivo. Second, Cas9 proteins are considered as exogenous antigens by the host immune system, which contributes to immune clearance and inflammatory responses, undermining therapeutic stability and efficacy. Third, off-target editing events and the low precision and efficiency of homology directed repair (HDR) make clinical gene therapy risky and dangerous. These three issues are highly connected with each other. Being versatile and multifunctional delivery tools, nanocarriers are capable of addressing delivery barriers, immune rejection, and editing inaccuracies by enabling targeted and controllable delivery of CRISPR/Cas9, validating them as promising solutions for clinical gene editing applications [8].

4. Nanocarrier-assisted gene editing for antitumour research

4.1. Lipid nanoparticle-mediated gene editing for antitumour therapy

Lipid nanoparticles (LNPs) are clinically corroborated, advanced delivery systems that enable the translational application of CRISPR/Cas9 technologies. They present favorable biocompatibility and amenability to large-scale manufacture. While LNPs prevent CRISPR editing components from enzymatic degradation, they also assist intracellular internalization via endocytosis, allowing effective delivery within primary lymphoid tissues and tumour lesions. In preclinical glioblastoma models, targeted delivery of CRISPR/Cas9 against the polo-like kinase 1 (PLK1) gene by means of LNPs achieves high effectiveness of gene editing in vivo, resulting in significant regression of tumours and prolonged survival of the animals treated [9].

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4.2. Polymeric nanocarrier-assisted gene editing for drug-resistant tumours

Polymeric nanocarriers display several remarkable characteristics, such as adjustable structural flexibility and high payload capacity for chemotherapeutic agents, which constitute an excellent solution for chemoresistant tumour therapy. Platforms with such features allow co-delivery of both CRISPR/Cas9 editing components and chemotherapy drugs, permitting targeted tumour accumulation in response to the stimuli of tumour microenvironment. For instance, polymeric nanocarriers encapsulating both CRISPR/Cas9 and paclitaxel can silence multidrug resistance-related genes to reverse tumour chemoresistance while exerting great cytotoxic effects on tumour cells to improve overall therapeutic efficacy [10].

4.3. Inorganic nanocarrier-mediated gene editing for synergistic multimodal therapy

Inorganic nanocarriers, including mesoporous silica nanoparticles, are increasingly exploited in the field of multimodal cancer therapies because of their unique mechanical properties and versatile surface modification abilities. Specifically, these nanoplatforms can co-deliver CRISPR/Cas9 systems along with either photothermal or chemotherapeutic agents, allowing for integrated gene editing and targeted treatment within a single delivery vector. These combined therapeutic approaches have a synergistic effect that boosts the general antitumour effectiveness through inhibiting the proliferation of tumour cells and activating systemic antitumour immune responses [11].

4.4. DNA nanoframework-based precise gene editing systems

The most widely applicable in tumour therapy is the glutathione (GSH) reduction-responsive DNA nano-framework, which enables controlled delivery of Cas9 ribonucleoprotein (Cas9-RNP). Acrylamide-modified DNA (acDNA) serves as an initiator to trigger hybridization chain reaction within the nano-framework. Through this reaction, guide RNA is integrated into the DNA nano-framework, forming an RNA-DNA complex; meanwhile, the internal cavity of the nano-framework expands, facilitating the loading of Cas9 protein. Relying on the sol-gel phase transition, the nano-framework swells below the minimum critical dissolution temperature, achieving high-capacity loading of Cas9 protein. This approach not only enables efficient loading of Cas9-RNP but also maintains the original biological activity of the Cas9 protein during the loading process. Bisacrylamide cystamine (BACA), containing disulfide bonds, is used as a cross-linker to achieve responsive release of Cas9-RNP. The GSH concentration in tumour cells is at least four times that of normal cells. Disulfide bonds respond to glutathione and break, which cause the nano-framework to completely disintegrate and release the internally encapsulated Cas9 protein. The highly expressed RNase H in tumour cells degrades the RNA component of the DNA-RNA complex, leading to controlled release of Cas9-RNP and subsequent completion of the gene editing process [12].

4.5. Biomimetic nanocarriers for long-term gene editing-mediated antitumour therapy

Biomimetic nanocarriers constructed from natural cell membranes or extracellular vesicles, including exosomes, microvesicles, apoptotic bodies, large tumour vesicles, and other sizes and categories. The surface and interior of this type of nanocarrier contain functional proteins derived from mother cells, inheriting the biological characteristics of primitive cells and regulating the physiological behavior of receptor cells. The carrier with this characteristic can evade immune

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system clearance, accurately accumulate in tumour-targeted areas, and achieve long-term circulation in the body [13].

5. Advantages and current bottlenecks of nanocarrier-assisted tumour gene editing

5.1. Core application advantages

Nanocarrier-assisted gene editing technology can improve the efficiency of cellular uptake and allow for specific accumulation in tumours. It can be used to co-deliver multiple components(such as gene editing plasmids and therapeutic agents), facilitate endosomal escape, and decrease negative immune responses. This flexible technology provides a reliable strategy for diverse therapeutic applications and creates stable antitumour effects when used in vivo. Naturally derived nanomaterials are especially biocompatible and have low systemic toxicity, making them appropriate for biological applications [1].

5.2. Technical and translational bottlenecks at the present stage

Currently, nanocarrier-based gene editing methods still have multiple challenges. Complicated in vivo biological environments hinder their clinical translation and penetration into deep-seated tumours. Moreover, it is difficult for a single nanocarrier to simultaneously achieve high loading capacity, circulatory stability, tumour targeting ability, and stimulus-responsive performance, which leads to batch-to-batch quality variation and insufficient gene editing efficiency for clinical cancer therapy [2].

6. Conclusion

This article systematically reviews the basic theories and preclinical antitumour applications of nanocarrier-assisted CRISPR gene editing and analyzes its core strengths and limitations based on available oncological data. It clarifies to researchers its unique merits compared with chemotherapy, radiotherapy, and viral gene delivery, as well as intractable tumour obstacles and unresolved technical bottlenecks.

Future research may focus on four priorities: boosting tumour-targeted delivery efficiency of CRISPR cargos; building multifunctional unified nanocarriers integrating the advantages of mono-material platforms; standardizing fabrication workflows to guarantee batch uniformity; and optimizing biosafety assessment to reduce off-target and immune risks. These advancements require a solid understanding of the in vivo microenvironment and the metabolic pathways of tumours. For the mass production of nano-gene drugs, manufacturers and research groups must ensure stringent quality control over the composition of carriers as well as active editing payloads to safeguard patient safety.

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