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Review Article
International Journal of Pharmacy and Engineering (IJPE)
ISSN 2320-849X

Nanotechnology based delivery of CRISPR/Cas9 for cancer treatment - overview, application & future perspective

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Abstract

Cancer is one of the most leading causes of death. Chemotherapy and radiation therapy comes with severe side effects. This also have some limitations. CRISPR/Cas9 technology is revolutionary in cancer therapy. Oncogenes and tumor suppressor genes can be modified precisely by this technique. Lipid nano-particles, polymeric nano-particles, gold nano-particles, exosomes are included in nano technology based delivery system. These techniques have a great potential in improving cellular uptake, reducing immune response and targeting efficiency. The specialty of this nano carriers are increased bio-compatibility, controlled released and reduced of target effects. But still immune reactions, tumor specific targeting is not properly addressed. Future research should focuse on developing smart nano carriers, stimuli responsive system and personalized therapy. So that gene editing can be done more precisely.

Key words: CRISPR/Cas9, nanotechnology, cancer therapy, gene editing, lipid nano particles, polymeric nano particles, targeted delivery of target effect, tumour suppressant, personalized medicine.

Introduction

Nearly 10 million deaths worldwide were attributed to cancer in 2020, with lung, colorectal, liver, and breast cancers being the most deadly types. Ageing populations, environmental variables, and changes in lifestyle are all contributing causes to the increased occurrence, which calls for immediate advancements in prevention, early identification, and treatment approaches [1]. Traditional cancer therapies have many drawbacks. Radiation and chemotherapy frequently have high toxicity, which damages healthy tissues and makes them resistant to treatment [2]. Personalized treatments are essential since immunotherapy, while its efficacy, is constrained by immune evasion mechanisms and varying patient responses [3]. By permitting precise alterations of oncogenes and tumor suppressor genes, gene editing technologies such as CRISPR-Cas9

have transformed cancer therapy and improved targeted treatments. Although issues like off-target effects and delivery techniques still exist, CRISPR-based strategies show promise in repairing mutations, enhancing immune cell therapies, and defeating drug resistance [4,5]. A guide RNA (gRNA) is used by the potent gene-editing technique CRISPR/Cas9 to steer the Cas9 endonuclease to a particular DNA sequence, causing double-strand breaks. This allows for precise alterations by base editing and prime editing, which prevent double-strand breaks, as well as gene knockout through non-homologous end joining (NHEJ), gene knock-in through homology-directed repair (HDR), and more [6]. Because CRISPR/Cas9 is quicker, more effective, less expensive, and simpler to build than previous gene-editing technologies like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), it can be used for both research and therapeutic purposes [7, 8]. Successful genome editing depends on effective CRISPR/Cas9 transport since ineffective delivery can result in immunological reactions, off-target consequences, and low editing effectiveness. In terms of effectiveness, safety, and tissue specificity, current approaches include physical techniques (electroporation, microinjection), lipid nanoparticles (LNPs), and viral vectors (AAV, lentivirus); each has pros and cons [9, 10]. Lipid nanoparticles (LNPs), polymeric nanoparticles, and gold nanoparticles are examples of nanotechnology-based delivery systems that increase CRISPR/Cas9 efficacy by facilitating targeted distribution, enhancing cellular absorption, and shielding the cargo from degradation. These technologies overcome the main drawbacks of viral and conventional delivery methods by reducing toxicity, off-target effects, and immune responses. [11]

A)CRISPR/Cas9 Delivery Strategies

- **Traditional Delivery Approaches –**

Viral vectors (AAV, lentivirus, adenovirus) - Because of their high efficiency and steady gene expression, viral vectors like lentivirus, adenovirus, and adeno-associated virus (AAV) are frequently utilized for CRISPR/Cas9 delivery. Although AAV has a limited cargo capacity, it is recommended because to its low immunogenicity. Because it integrates into the host genome, lentivirus raises safety concerns yet is beneficial for long-term expression [12]. Adenoviruses have a greater cargo capacity and offer transitory expression, but they can also elicit powerful immunological reactions. However, obstacles including limited cargo size (AAV), immunogenicity (adenovirus), and insertional mutagenesis (lentivirus) prevent them from being widely used in clinical settings, requiring better vector engineering [13].

Physical methods (electroporation, microinjection, hydrodynamic injection) - Physical methods, such as **electroporation, microinjection, and hydrodynamic injection**, enable direct CRISPR/Cas9 delivery into target cells without relying on viral or chemical carriers. **Electroporation** uses electrical pulses to transiently open cell membranes, allowing gene editing components to enter, but can cause cell damage and low viability [14]. **Microinjection** offers

high precision by directly injecting CRISPR/Cas9 into individual cells, making it ideal for embryos and stem cells, though it is labor-intensive and low-throughput. **Hydrodynamic injection**, mainly used in animal models, involves rapid injection of CRISPR/Cas9 solutions into the bloodstream, achieving high liver transfection but causing transient tissue damage [15].

B)Nanotechnology-Based Delivery Approaches

Advantages of nanocarriers for CRISPR/Cas9 delivery - When it comes to CRISPR/Cas9 distribution, nanocarriers have a number of benefits that increase the treatment's potential for cancer [16]. They guarantee accurate gene editing with the least amount of off-target consequences by offering high biocompatibility, decreased immunogenicity, and improved cellular absorption [17]. Furthermore, by facilitating stimuli-responsive release, nanocarriers enhance therapeutic efficacy and shield CRISPR components from deterioration. Their adaptability enhances specificity and lowers systemic toxicity by enabling tailored administration [18].

- Types of nanocarriers used:
    - **Lipid-based nanoparticles (LNPs) -** PEGylated liposomes and cationic lipids are widely used for CRISPR/Cas9 delivery due to their **enhanced circulation time, reduced immunogenicity, and efficient cellular uptake [19]**. PEGylation prevents rapid clearance by the immune system, while cationic lipids **facilitate endosomal escape and improve gene editing efficiency [20]**. These lipid-based carriers provide a biocompatible and tunable platform for precise CRISPR delivery in cancer therapy [21]

FDA-approved LNPs for mRNA vaccines (potential adaptation for CRISPR - A promising platform for CRISPR/Cas9 delivery is offered by FDA-approved lipid nanoparticles (LNPs), which are extensively utilized in mRNA vaccines such as Pfizer-BioNTech and Moderna COVID-19 vaccines. These LNPs reduce immunological responses while improving nucleic acid stability, cellular uptake, and controlled release. Because of their demonstrated safety and effectiveness in clinical settings, researchers are actively investigating how they might be modified for CRISPR-based gene editing treatments [22].

Polymeric nanoparticles - Effective nanocarriers for regulated CRISPR/Cas9 release that increase gene-editing accuracy include PLGA, chitosan, and dendrimers [23]. While chitosan improves cellular absorption and endosomal escape, PLGA provides biodegradability and prolonged release [24]. Because of their high gene-loading capacity and branching structure, dendrimers facilitate effective intracellular CRISPR delivery, minimizing off-target effects and enhancing therapeutic results [25].

Inorganic nanoparticles - Because of its photothermal effects, which allow for regulated gene editing activation by laser irradiation, gold nanoparticles (AuNPs) have drawn attention in the delivery of CRISPR/Cas9 [26]. AuNPs reduce off-target effects while improving CRISPR

component cellular absorption, stability, and targeted release [27]. This photothermal-triggered method presents a viable precision cancer treatment option [28]

Mesoporous silica nanoparticles (MSNs) for tumor targeting - Mesoporous silica nanoparticles (MSNs), which have a high drug-loading capacity, biocompatibility, and controlled release, are promising vehicles for tumor-targeted CRISPR/Cas9 delivery [29]. Tumor-targeting ligands can be added to functionalized MSNs to increase cellular uptake and selectivity [30]. Because of their porous nature, CRISPR cargo may be efficiently encapsulated, guaranteeing accurate gene-editing applications in cancer therapy [31]

Exosome-based delivery - CRISPR/Cas9 distribution via exosomes provides a biocompatible and immune-evasive method of cancer treatment. CRISPR components are well encapsulated by these naturally occurring extracellular vesicles, allowing for precise distribution with little immunological reaction. They are a promising treatment option for cancer because of their capacity to transcend biological barriers, which improves the accuracy of gene editing and therapeutic efficacy. [32]

Peptide-based delivery systems - Peptide-based delivery systems utilize **cell-penetrating peptides (CPPs)** to enhance cellular uptake of CRISPR/Cas9, ensuring efficient gene editing. These CPPs facilitate **non-toxic membrane penetration and endosomal escape**, improving intracellular delivery. Their tunability allows for **targeted and controlled CRISPR release**, making them a promising strategy for cancer therapy. [33]

C)Applications in Cancer Therapy

Gene Knockout for Oncogene Suppression - Because CRISPR-mediated gene knockdown targets important cancer drivers like KRAS, MYC, TP53, EGFR, and BRAF, it has demonstrated considerable potential in oncogene suppression [34]. By inhibiting these oncogenes, cancer cells become more sensitive to treatments, tumor development is disrupted, and apoptosis is enhanced [35]. This method offers an accurate, flexible approach to cancer treatment that is tailored to each patient [36].

Gene Correction & Activation of Tumor Suppressor Genes - A potent cancer treatment approach is provided by CRISPR-based gene repair and the activation of tumor suppressor genes like TP53, PTEN, and BRCA1/2 [37]. Restoring the functioning of these genes can improve DNA repair, trigger apoptosis in cancer cells, and restart cell cycle arrest [38]. Precision medicine could greatly benefit from this strategy, especially for malignancies caused by mutations in these important regulators [39]

Base Editing & Prime Editing in Cancer - Advanced CRISPR-based technologies called base editing and prime editing allow for the accurate repair of mutations linked to cancer without causing double-strand breaks [40]. Prime editing permits controlled insertions, deletions, and replacements, whereas base editing modifies single nucleotides directly [41]. These methods

offer a safer and more effective way to fix genes, which makes them very promising for cancer treatment [42].

CRISPR for Overcoming Drug Resistanc - CRISPR technology targets important resistance genes like EGFR-T790M and ABCB1 to overcome treatment resistance in cancer [43]. Restoring drug sensitivity, improving the effectiveness of chemotherapy and targeted therapy, and lowering tumor recurrence are all achieved by knocking out or correcting these genes [44]. For treating therapy failure, this method presents a promising precision medicine option [45].

CRISPR in Cancer Immunotherapy - By altering immune checkpoint genes like PD-1, PD-L1, and CTLA-4, CRISPR is transforming cancer immunotherapy and boosting T-cell responses against malignancies. By altering these checkpoint pathways, one can improve T-cell infiltration, raise anti-tumor immunity, and increase the effectiveness of checkpoint inhibitor therapy all of which open up new possibilities for individualized cancer treatment[46]. By enhancing target selectivity, persistence, and immune evasion, CRISPR-engineered CAR-T cells improve precision cancer treatment [47]. These changes improve the effectiveness of tumor-killing, lessen fatigue, and develop universal CAR-T treatments [48]. CRISPR makes it possible to precisely modify immune checkpoint genes (PD-1 deletion, for example) to improve T-cell longevity and function, increasing the efficacy of CAR-T treatments against hematologic and solid cancers [49].

CRISPR for Cancer Stem Cells (CSCs) Elimination - By specifically targeting markers like CD44, ALDH1, and OCT4, which are essential for CSC self-renewal and tumor growth, CRISPR technology makes it possible to eradicate cancer stem cells (CSCs). By reducing tumor initiation, metastasis, and drug resistance, disrupting these genes is a viable strategy for eliminating CSCs and averting cancer patients' relapses. [50]

Synergistic Approaches - To improve the effectiveness of cancer treatment, CRISPR-based gene editing is being used in conjunction with RNA-based medicines [51], photodynamic therapy (PDT), and photothermal therapy (PTT) [52]. A highly tailored and minimally invasive therapeutic approach is provided by this synergistic technique, which enables precision gene editing while also inducing tumor cell killing by light-activated or RNA-modulated processes [53].

D) Challenges and Limitations

- **Off-Target Effects & Genetic Instability**

Although CRISPR/Cas9 technology has transformed gene editing, cancer treatments are seriously threatened by the potential for unintended genetic alterations caused by its off-target effects. [54] SpCas9-HF, eSpCas9, and Cas12 are examples of modified Cas enzymes that have been created to increase target selectivity and reduce off-target effects. [55] Cas13 also has RNA-targeting properties, which improves gene editing accuracy even more.[56]

- **Immune Responses & Toxicity**

Because Cas enzymes are bacterial, CRISPR/Cas9-based cancer treatments can elicit immune responses. [57] The results of treatment may be impacted by immunological rejection or inflammatory responses. [58] Furthermore, systemic toxicity can be caused by delivery systems that use viral vectors or nanoparticles, which raises questions regarding patient safety and the therapy's long-term effects. [59]

- **Nanocarrier Limitations**

Because of their ability to protect nucleic acids and their biocompatibility, nanocarriers such as liposomes, dendrimers, and polymeric nanoparticles are frequently utilized for the delivery of CRISPR/Cas9. [60] Effective distribution is, however, severely hampered by their low transfection efficiency, poor stability in vivo, and restricted tissue penetration. [61] Furthermore, some nanocarriers have the potential to generate cytotoxicity or immunological responses, which would decrease their therapeutic efficacy. [62]

- **Tumor-Specific Delivery Issues**

One of the biggest obstacles in cancer treatment is delivering CRISPR/Cas9 to specific tumors because of the variety of tumor microenvironments, the absence of suitable biomarkers, and the off-target distribution to healthy tissues. [63] Although lipid nanoparticles and exosomes are examples of nanocarriers that have demonstrated potential, their effectiveness in targeting tumors is still restricted. [64,66] To increase tumor selectivity, surface modification techniques are being investigated, such as ligand-based targeting. [65]

- **Scalability and Clinical Translation Barriers**

Batch-to-batch variability, high manufacturing costs, and a lack of established methods are obstacles to the scalability and clinical translation of CRISPR/Cas9-based nanomedicine. [67,69] A major obstacle to the widespread use of these delivery methods in clinical settings is the challenge of scaling up nanoparticle manufacturing while maintaining uniform quality. [68] Furthermore, regulatory obstacles cause clinical implementation to be further delayed. [70]

E) Future Perspectives & Clinical Translation

- **Next-Generation CRISPR Delivery Platforms**

  - **Smart nanocarriers for controlled release**

To improve the effectiveness, accuracy, and safety of CRISPR/Cas9-based cancer treatments, next-generation CRISPR delivery platforms have been developed. [71] Biodegradable polymers, exosome-based systems, and lipid nanoparticles (LNPs) are examples of these platforms, which enhance cellular absorption while reducing off-target effects. [70,71] Moreover, hybrid delivery techniques that combine viral vectors and nanoparticles are being investigated to get around the drawbacks of conventional delivery methods. [72]

- **Stimuli-responsive nanoparticles (pH, redox, enzyme-sensitive)**

The distribution of CRISPR/Cas9 systems is improved by stimuli-responsive nanoparticles, which react to certain tumor microenvironmental cues such pH variations, redox gradients, and enzyme activity. [73,75] These nanoparticles enhance intracellular absorption and targeted release, decreasing off-target effects and boosting gene editing systems' therapeutic efficacy. [74,76]

➢ Personalized CRISPR Therapy for Cancer Patients

With personalized CRISPR therapy, gene-editing therapies are more accurate and effective since they can be customized to each cancer patient's own genetic composition. [77] With this method, patient-specific mutations can be fixed or immune cells can be altered to improve anti-tumor efficient operation. [78] Clinical translation is still hindered by issues with delivery methods, regulatory authorization, and genetic heterogeneity appraisal. [79]

➢ Advancements in In Vivo Gene Editing

Cancer treatments are now far more accurate and effective thanks to recent developments in in vivo CRISPR/Cas9 gene editing. [79,80] Novel delivery methods, like viral vectors and lipid nanoparticles (LNPs), allow for the precise delivery of gene-editing tools to tumors. [81] Furthermore, gene alterations in living tissues may now be made more precisely and with less disruption thanks to novel methods like prime editing and base editing. [82]

➢ Combination Therapies

Combination medicines that combine CRISPR/Cas9 with traditional cancer treatments such as immunotherapy, radiation, and chemotherapy provide synergistic effects that improve treatment results. [83] By altering radiation susceptibility pathways, increasing the curative effects of immune checkpoint inhibition, or sensitizing tumors to chemotherapy, CRISPR/Cas9 can increase the susceptibility of cancer cells to conventional treatments. [84]

- **CRISPR + checkpoint inhibitors**

By increasing T-cell activity and overcoming resistance mechanisms, the combination of CRISPR/Cas9 and immune checkpoint inhibitors (ICIs) boosts cancer immunotherapy. [84,85] By erasing inhibitory genes like CTLA-4 and PD-1, CRISPR can make cancer cells susceptible to immune checkpoint inhibition. [86] Although this synergistic method needs to be further enhanced for clinical translation, it shows promise in treating resistant cancers. [87]

- **CRISPR + RNA-based therapies (siRNA, miRNA)**

By focusing on both genetic alterations and post-transcriptional gene control, CRISPR/Cas9 in conjunction with RNA-based therapeutics like siRNA and miRNA improves the treatment of cancer. A multifaceted treatment approach is provided by CRISPR/Cas9, which can alter the

genes that cause cancer, and siRNA or miRNA therapy, which can either silence oncogenes or restore tumor-suppressive miRNA functions. [88]

- **Regulatory & Ethical Considerations**

The possibility for germline editing, informed consent, and genetic privacy are only a few of the serious ethical and regulatory issues brought up by the clinical use of CRISPR/Cas9 for cancer treatment. [88,89] Globally, there are differences in regulatory systems; in many places, the use of gene editing in humans is restricted by stringent laws. [89] The main goal of ethical considerations is to weigh the hazards of accidental modifications to genes against the therapeutic possibilities. [89,90]

- **Guidelines for CRISPR-based cancer treatments**

Clinical trials involving gene editing must be safe, effective, and ethically transparent, according to international standards for CRISPR-based cancer treatments. [91] Important elements include long-term tracking of genetic changes, patient informed consent, and standardized procedures for off-target evaluation. [92] The FDA and EMA are among the regulatory agencies that are gradually creating frameworks to control gene-editing treatments. [93]

- **Long-term safety concerns and ethical debates**

Immune responses, genomic instability over time, and possible off-target mutations are the main causes of long-term safety issues with CRISPR/Cas9-based cancer treatments. [94] The irreversible modification of human genomes—especially in germline editing—raises ethical concerns around genetic enhancement, consent, and fair access to these treatments. [95,96]

F) Conclusion:

By overcoming significant obstacles such inadequate cellular absorption, immune clearance, and off-target consequences, nanotechnology has completely changed the way CRISPR/Cas9-based cancer medicines are delivered. [97] The effective encapsulation and preservation of CRISPR components by nanocarriers such as lipid nanoparticles (LNPs), polymeric nanoparticles, dendrimers, and gold nanoparticles improves their circulation time and delivery to target cells. [98] In order to ensure site-specific gene editing, stimuli-responsive nanoparticles can also release the CRISPR payload in response to variables in the tumor microenvironment, such as pH, redox potential, or enzyme activity. [99] The precision of gene editing and biocompatibility are further enhanced by hybrid nanocarrier systems that combine various materials. [100] Nanotechnology is a viable approach for upcoming cancer gene-editing medicines since it can offer non-viral, targeted delivery systems. [101] Clinical translation, long-term safety, and scalability are still major obstacles. [102]

Since precision nanomedicine makes it possible for targeted drug delivery, real-time tumor monitoring, and customized cancer therapy, it is quickly becoming a game-changing technique in oncology. [103,105] Drugs or gene-editing tools can be delivered directly to tumor cells by nanocarriers including metal nanoparticles, dendrimers, and lipid nanoparticles (LNPs), reducing systemic toxicity. [104] Additionally, therapeutic drugs can be released by stimuli-responsive

nanoparticles according to the tumor microenvironment, improving the accuracy of treatment. [105] By providing more precise gene-specific changes, the combination of CRISPR/Cas9 with nanomedicine is expanding the possibilities for individualized cancer treatment. [106]

G) Future outlook: overcoming current barriers for clinical translation

The creation of more accurate, secure, and scalable delivery methods is essential for the future of cancer treatment based on CRISPR/Cas9 nanotechnology. [107] It is anticipated that developments in cell-derived vesicles, biodegradable polymers, and stimuli-responsive nanoparticles will enhance targeted delivery and reduce off-target effects. [106,108] The accuracy of gene editing will be significantly increased by combining CRISPR with artificial intelligence (AI) for target site prediction and optimization. [109] Furthermore, by providing highly targeted gene-editing methods, personalized nanomedicine platforms that are customized to each patient's genome have the potential to completely transform cancer treatment. [110] To obtain regulatory approval, long-term monitoring of immunological reactions, mutagenesis hazards, and genetic stability is still necessary. [111,112] For CRISPR-based medicines to be translated into practical applications, cooperation between researchers, clinicians, and legislators will be essential to overcoming ethical, legal, and technological obstacles. [112,113]

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Received: May 15th 2025, Accepted: May 28th 2025