A Narrative Review of the Research Advances in CRISPR/Cas9 for the Treatment of Solid Tumors

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Abstract: The CRISPR-Cas9 gene editing technology offers a revolutionary method for addressing drug resistance and heterogeneity in solid tumors; however, its clinical use remains limited by inefficient in vivo delivery. This review systematically summarizes advances in this field, focusing on innovative strategies to overcome delivery bottlenecks. Firstly, the article outlines the mechanism of action of CRISPR system and development of novel tools like base editors and prime editors. Subsequently, it has a detailed comparison of the advantages, limitations, and latest applications of viral vectors, non-viral nanocarriers (including lipid nanoparticles, polymers, mesoporous silica, etc.), and physical delivery methods. Furthermore, the review integrates and analyzes the therapeutic exploration of CRISPR technology in six major types of solid tumors (non-small cell lung cancer, triple-negative breast cancer, colorectal cancer, hepatocellular carcinoma, glioblastoma, and pancreatic ductal adenocarcinoma). It covers various strategies which demonstrate preclinical tumor-suppressive effects. Finally, the review discusses the major challenges currently faced, such as delivery efficiency, off-target effects, and tumor heterogeneity. Additionally, future directions of intelligent delivery systems, high-fidelity editing tools, and personalized combination therapies are prospected.

Keywords: CRISPR/Cas9; Solid Tumors; Gene Editing; Drug Delivery Systems; Nanocarriers; Preclinical Studies; Tumor Microenvironment

1. Introduction

Cancer is one of the leading causes of death worldwide, with solid tumors-such as lung, breast, colorectal cancers-accounting for approximately 90% of cases [1]. Although traditional treatment methods like surgery, chemotherapy, and radiation therapy benefit some patients, their effect is often limited by severe toxicity and drug resistance. Targeted therapies and immunotherapies have offered new promise, yet challenges such as low response rates and tumor heterogeneity persist.

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 gene-editing technology has high efficiency and precision. Feng Zhang's team first used it in precise genome editing in mammalian cells, which provided a revolutionary tool for cancer therapy[2]. It enables modification of key oncogenes like KRAS [3] and EGFR [4], and the immune checkpoints such as PD-1 and CTLA-4 [5], thereby inhibiting tumor growth or enhancing antitumor immunity. However, applications in solid tumors face delivery challenges, off-target effects, and tumor microenvironment suppression [6]. Overcoming these obstacles requires developing safe, efficient, and tumor-specific CRISPR delivery systems capable of controllable editing.

It further analyzes delivery system design, strategies to overcome physiological barriers (e.g., dense stroma, the blood-brain barrier, and nonspecific distribution), and mechanisms enhancing therapeutic outcomes. Current technical limitations and future directions are also discussed.

2. Methods

2.1 Literature Search Strategy

PubMed and Web of Science were searched from January 2013 to March 2025 using the following string:(CRISPR OR "CRISPR-Cas9") AND ("solid tumor" OR "solid tumor" OR carcinoma* OR sarcoma* OR NSCLC OR TNBC OR CRC OR HCC OR GBM OR PDAC) AND (deliver* OR "drug delivery" OR nanocarrier* OR "lipid nanoparticle" OR "viral vector" OR AAV OR electroporation)

The search was limited to English-language articles published in peer-reviewed journals. Additionally, reference lists of highly cited papers and recent expert reviews were manually screened to capture studies that might have been missed by the electronic search.

2.2 Study Selection and Eligibility Criteria

Studies were included if they (i) employed CRISPR/Cas9 or its derivatives (base/prime editors), (ii) focused on solid-tumor models (in vitro, in vivo, or ex vivo), and (iii) reported experimental data on delivery systems. Reviews, conference abstracts, commentaries, and studies limited to hematologic malignancies were excluded. Titles and abstracts were first assessed by one author; uncertainties were resolved after discussion with the supervisor. Because this is a narrative rather than a systematic review, no PRISMA flow diagram or formal risk-of-bias assessment was performed.

2.3 Data Extraction and Thematic Synthesis

Eligible articles were read in full, and the following information was extracted: author, year, tumor model, CRISPR format, delivery platform, administration route, main findings, and limitations. Studies were then thematically grouped first by delivery platform (viral, non-viral nanocarrier, physical, and emerging hybrid systems) and second by the six major solid-tumor types covered in this review (NSCLC, TNBC, CRC, HCC, GBM, PDAC). This approach yields a comprehensive, forward-looking overview while acknowledging that the included literature is representative but not exhaustive.

3. The Mechanism of the CRISPR/Cas9 System

The function of the CRISPR/Cas9 comes from the adaptive immune mechanism of bacteria against bacteriophages and operates through the synergistic action of guide RNA (gRNA) and Cas9 nuclease. gRNA is made by the fusion of CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA): the 5'-end of crRNA recognizes the target DNA sequence through base complementary pairing, while the 3'-end stem-loop of tracrRNA binds to Cas9 to form a ribonucleoprotein (RNP) complex with catalytic activity. Targeting specificity relies on the recognition of the protospacer adjacent motif (PAM): SpCas9 derived from Streptococcus pyogenes only cleaves DNA sites adjacent to 5'-NGG-3'. Upon binding target DNA, Cas9 activates its nuclease domains: the HNH domain cleaves the gRNA-complementary strand, and the RuvC domain cleaves the non-complementary strand, resulting in double-strand breaks (DSBs) [5]. Cells repair DSBs through two conserved pathways: Non-homologous end joining (NHEJ) is an error-prone mechanism that directly ligates broken ends and often introduces random insertions or deletions (indels), leading to gene knockout. Homology-directed repair (HDR) needs a donor DNA template and enables precise gene replacement via Rad51-mediated strand invasion, but efficiency is typically below 10% and restricted to dividing cells.

The applicability and precision of the CRISPR system continue to improve. Variants such as SaCas9-KKH (recognizing 5'-NNNRRT-3' PAM) [7] and Cpf1 (generating sticky ends) [8], have expanded targetable genomic regions. Base editors like ABEs avoid DSBs achieving efficient AT-to-GC conversion with ≤0.1% indel rates [9]. Prime editors use nCas9-reverse transcriptase fusion proteins and engineered pegRNAs [10].

4. Delivery Strategies for CRISPR/Cas9 in Solid Tumors

The therapeutic efficacy of the CRISPR/Cas9 system relies on the ability to deliver functional CRISPR components (Cas nucleases and guide RNAs) into the nuclei of target tumor cells. The complex in vivo barriers of solid tumors-such as the dense extracellular matrix, high interstitial fluid pressure, and immune surveillance-pose notable challenges to this process. Delivery strategies are mainly classified into two major types: viral vectors and non-viral vectors, each with unique advantages and limitations.

4.1 Viral Vectors

Viral vectors, including adenoviruses (AdV), adeno-associated viruses (AAV), lentiviruses (LV), are highly efficient delivery tools. Studies have shown that the delivery of plasmids encoding CRISPR components to the pancreas of mice via hydrodynamic transfection can successfully generate a tumor model that is highly similar to human pancreatic ductal adenocarcinoma (PDAC) in both histological and molecular characteristics, which demonstrates the efficient in vivo delivery

capability of AAV. However, clinical use of viral vectors remains limited by their constrained packaging capacity (especially AAV), immunogenicity, and risk of insertional mutagenesis.

4.2 Non-Viral Nanocarriers

Non-viral vectors, especially nanocarriers, are attracting many attentions due to their advantages such as low immunogenicity, high loading capacity, scalable production, and easy surface functional modification. They mainly include lipid nanoparticles (LNPs), polymeric nanoparticles, and inorganic nanoparticles.

4.2.1 Lipid nanoparticles (LNPs)

LNPs are currently the most advanced non-viral delivery platforms. In lung cancer treatment, studies have shown that through optimized formulations and delivery parameters, LNPs can effectively encapsulate and deliver CRISPR components. For example, delivery of a CRISPR system targeting the KRAS G12S mutation via electroporation achieved nearly 50% editing efficiency in in vivo models, greatly inhibiting tumor growth [12].

4.2.2 Polymeric nanoparticles

Cationic polymers (e.g., polyethyleneimine, PEI) can compress negatively charged CRISPR components into complexes through electrostatic interactions. Research used cationic polymer vectors to mediate the specific editing of the EGFR T790M allele. By introducing surface charge engineering and nuclear localization signal (NLS) peptides, it successfully addressed the in-vivo stability issue of nucleases, improved nuclear delivery efficiency, and thereby reversed targeted therapy resistance [11].

4.2.3 Inorganic nanoparticles & smart responsive vectors

Inorganic materials (e.g., mesoporous silica) and intelligently designed vectors exhibit great potential in targeting ability and environment-responsive release. The Wan team [20] developed a pH-responsive dual-target CRISPR ribonucleoprotein (RNP) nanocarrier that remains electrically neutral in blood circulation to minimize nonspecific adsorption, then switches to positive charge in the acidic tumor microenvironment to enhance cellular binding and endocytosis. This system achieved simultaneous editing of APC and KRAS genes in colorectal cancer with 65% efficiency and significantly suppressed xenograft tumor growth. Similarly, Zhang et al. [26] constructed targeted hollow mesoporous organosilica nanoparticles (HMONs) to co-deliver sorafenib and EGFR-targeting CRISPR/Cas9, yielding a tumor inhibition rate of 82.5% through synergistic chemo-gene therapy in liver cancer models.

4.3 Physical Delivery Methods

Physical methods achieve cytoplasmic delivery by temporarily disrupting the cell membrane barrier through physical means.

Electroporation uses control electrical pulses to make transient pores in the cell membrane, enabling cytoplasmic delivery of macromolecules such as ribonucleoprotein (RNP) complexes. In clinical applications, this method has achieved over 90% PD-1 gene editing efficiency in T cells in treating NSCLC [14].

4.4 Other Emerging Strategies

Other innovative strategies continue to emerge. For instance, Liu et al. [31] employed engineered extracellular vesicles (EVs) to deliver a CRISPR/Cas9 system that knocks out GPX4, a key ferroptosis suppressor in glioblastoma, effectively inducing tumor ferroptosis and offering a radiosensitization strategy.

In summary, choosing a delivery strategy depends on the application and therapeutic objectives. Viral vectors offer high efficiency but carry safety risks; non-viral nanocarriers provide design flexibility and improved safety, making them a major research focus; physical methods are effective for ex vivo editing, like chimeric antigen receptor T-cell (CAR-T) preparation.

5. The Applications of CRISPR/Cas9 in Major Types of Solid Tumors

5.1 Non-Small Cell Lung Cancer (NSCLC)

Lung cancer ranks first among cancer-related deaths globally, with NSCLC accounting for approximately 85% of all cases. EGFR mutations exceed 40% in Asian populations, while the KRAS-driven mutations surpass 30% in Western populations [1]. The 5-year survival remains below 20%, largely due to acquired resistance and the immunosuppressive tumor microenvironment (TME).

CRISPR/Cas9 solves this therapeutic dilemma through targeted gene editing and TME remodeling. In targeted therapy: Cationic polymer vectors mediate allele-specific editing of the EGFR T790M mutation, reversing drug resistance. Its innovation lies in the use of

surface charge engineering to overcome the core challenge of nuclease stability in vivo, while the introduction of nuclear localization signal (NLS) peptides greatly improves nuclear delivery efficiency. In immunotherapy: Standardized electroporation platforms have achieved >90% PD-1 gene editing efficiency in T cells during clinical translation [14]. After treatment, 12 drug-resistant patients had an objective response rate of 41.7%. And in terms of safety, only 1 patient had grade 2 cytokine release syndrome (CRS). PD-1 gene editing in T cells restores its cytotoxic function by relieving endogenous immune suppression and remodels the composition of the tumor immune microenvironment [11, 14].

Electroporation overcomes the "undruggable" KRAS barrier. Targeting KRAS G12S achieved nearly 50% editing efficiency in vivo, suppressed MAPK signaling, induced cell cycle arrest, reduced lung metastatic nodules by 82.4%, and extended median survival of tumor-bearing mice from 26 to 54 days (orthotopic lung cancer model, n = 10 per group). Furthermore, the COX2/PGE2 immunosuppressive axis identified through genome-wide screening reveals the molecular link between KRAS mutations and immune evasion, providing a novel target for combination therapies [12]. For example, to address TME suppression, Boumelha's team found that KRAS mutations activate the PTGS2 gene, which increases the secretion of prostaglandin E2 (PGE2) [13]. Based on this, a combination strategy was developed using CRISPR to knockout the PTGS2 gene, resulting in a 76.5% reduction in PGE2 secretion. This strategy improved the tumor inhibition rate of anti-PD-1 therapy, with a 78.9% reduction in distant metastases.

5.2 Triple-Negative Breast Cancer (TNBC)

Triple-negative breast cancer (TNBC) is the most invasive and therapeutically challenging subtype of breast cancer. It constitutes around 15–20% of all breast cancer cases but contributes to over 25% of breast cancer-related deaths; the median survival of patients after metastasis is only 13 months. Standard chemotherapy often fails due to cancer stem cells (CSCs), impaired DNA repair, and a dense extracellular matrix, leading to high recurrence. CRISPR/Cas9 technology offers novel multi-mechanism interventions for TNBC.

To target the therapy-resistant cancer stem cell population, Wang et al. developed a core-shell nanocarrier system, where the hydrophobic core encapsulates doxorubicin, and the cationic shell compresses CRISPR/Cas9 plasmids to target the FBXO44 gene. The knockout of this gene significantly inhibits the self-renewal capacity of cancer stem cells. This co-delivery system achieves simultaneous killing of proliferating cells and CSC populations in vivo, resulting in a 72.3% reduction in tumor volume [15].

In epigenetic regulation: Mao et al. used CRISPR to knockout the EZH2 gene and found that this knockout relieves the silence of the tumor suppressor gene AXIN2 by reducing the level of H3K27me3 modification. This further inhibits the activity of the Wnt/β-catenin signaling pathway, leading to a 68.5% decrease in cell migration ability [16].

For tumors with deficient DNA repair, Mintz et al. introduced BRCA1 mutations via CRISPR, confirming that PARP1 editing combined with platinum drugs synergistically enhanced apoptosis by 4.2-fold through synthetic lethality [17]. Furthermore, to cope with metastasis, Yang et al. demonstrated that dual knockout of CXCR4/CXCR7 disrupted the TGF-β/Smad positive feedback loop, reducing lung metastasis nodules by 78.9% and offering a new strategy to inhibit pre-metastatic niche formation [18].

5.3 Colorectal Cancer (CRC)

Colorectal cancer (CRC) is the third most common cancer globally. Over 80% of cases involve APC inactivation, driving constitutive Wnt/β-catenin activation, while ~ 40% carry KRAS mutations sustaining MAPK signaling-together promoting proliferation and invasion [1,19]. Advanced CRC has a 5-year survival below 20% after liver metastasis.

The CRISPR/Cas9 technology has greatly advanced CRC mechanism research and the innovation of therapeutic strategies:

CRISPR/Cas9 has significantly advanced CRC research and therapy. Takeda et al. introduced APC, KRAS, and TP53 mutations into intestinal organoids using CRISPR, recapitulating adenoma-to-carcinoma progression and confirming β-catenin nuclear translocation as an early driver event [19]. Through genome-wide screening, Wan et al. identified USP47 as a novel Wnt pathway regulator; its loss reduced β-catenin stability, and combining a USP47 inhibitor with irinotecan synergistically increased apoptosis 3.5-fold ($p<0.01$) [20]. In the

development of targeted therapy, the innovation in delivery systems has become key to overcoming physiological barriers. The Wan team designed a pH-responsive dual-target CRISPR ribonucleoprotein (RNP) nanocarrier, which is electrically neutral in the neutral blood environment to reduce non-specific adsorption. Upon reaching the acidic tumor microenvironment, it switches to a positive charge, enhancing cell membrane binding and endocytosis efficiency. This enables simultaneous editing of the APC and KRAS genes, with an editing efficiency of up to 65%, effectively inhibiting the growth of xenograft tumors [21]. And in deciphering drug resistance mechanisms, Yu et al. identified the adaptor protein GRB7 as a key mediator of MEK inhibitor resistance through CRISPR loss-of-function screening. Knockout of GRB7 blocks the feedback reactivation of the ERK/FAK signaling pathway, improving the effect of MEK inhibitors by 4-fold and reducing tumor volume by 61.8% [22].

5.4 Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma (HCC) is the third cause of cancer deaths, and over 800,000 deaths annually, its 5-year survival rate is below 18%. Currently, the objective response rates of first-line systemic therapeutic drugs, such as multi-kinase inhibitors like sorafenib and immune checkpoint inhibitors like anti-PD-1 and CTLA-4 antibodies, are generally low (usually below 30%). This therapeutic dilemma arises from the high heterogeneity of HCC, the strongly immunosuppressive microenvironment, and the frequently accompanying cirrhotic background. The latter leads to excessive deposition of extracellular matrix, forming physical and biological delivery barriers.

CRISPR-based screens are instrumental in identifying key resistance mechanisms to targeted therapies in HCC. Wei et al. found phosphoglycerate dehydrogenase (PHGDH) is the key metabolic gene mediating the resistance of HCC cells to sorafenib. Its knockout disrupted serine metabolism and reduced sorafenib IC_{50} by 4-fold [23]. Similarly, Genome-wide screening also revealed novel mechanisms of lenvatinib resistance [24]. Beyond multi-kinase inhibitors, the same method used by Qing Li's team found that TRIM34 also acts as a key regulator of ferroptosis resistance in HCC, targeting TRIM34 enhances ferroptosis sensitivity, so developing inhibitors against it in combination with PD-L1 immunotherapy holds potential to overcome immunotherapy resistance [25].

The translational potential of CRISPR extends beyond target discovery to synergistic therapeutic strategies. Zhang et al. co-delivered sorafenib and EGFR-targeting CRISPR using hollow mesoporous organosilica nanoparticles (HMONs) , this increased sorafenib sensitivity 3.8-fold and achieved 82.5% tumor inhibition [26].

In terms of delivery technology, Yin et al. created an ultrasound-controlled CRISPR/Cas9 system, combining sonodynamic therapy (SDT) with gene editing. Ultrasound can activate sonosensitizers to generate reactive oxygen species (ROS) for tumor killing and precisely control the release of CRISPR components. This system achieved 65.4% gene knockout[27] and combined physical and gene therapy.

5.5 Glioblastoma (GBM)

Glioblastoma (GBM) is the most dangerous primary brain tumor in adults, the median survival of it is under 15 months. Key challenges include the blood-brain barrier (BBB), tumor heterogeneity, treatment resistance. CRISPR/Cas9 technology is addressing these through innovative strategies.

Overcoming the delivery barrier of the central nervous system, Zou et al. developed a CRISPR/Cas9 nanocapsule that effectively crosses the BBB, achieving over 40% editing efficiency, significantly inhibiting tumor growth and prolonging median survival of intracranial GBM-bearing mice increased by approximately 1.9-fold (Kaplan–Meier analysis, n = 8) in orthotopic GBM mouse model[28]. In resistance mechanism research, MacLeod et al. used genome-wide CRISPR screening to identify genes SOX2, OLIG2, maintaining GSC stemness and DNA repair. Knockout increased TMZ sensitivity by 3-5 times [29]. Using a similar method, the Huang team revealed the NF-κB/E2F6 axis mediates TMZ resistance in EGFRvIII-mutant GBM.

Combining CRISPR with immunotherapy shows strong promise. Choi et al. generated EGFRvIII-targeted CAR-T cells with PD-1 knockout in T cells. The killing activity of these edited CAR-T cells against tumor cells expressing EGFRvIII was increased by 2.5 times in vitro, and showed tumor clearance ability and a more durable anti-tumor effect in

tumor-bearing mouse models [30]. The study by Fierro Jr et al. provided another idea: they used CRISPR to directly knock out the PD-L1 gene in U87 glioma cells, which reversed the polarization of tumor-associated macrophages (TAMs) to anti-tumor M1 phenotype, improving the tumor microenvironment. In addition, CRISPR can develop new synergistic treatment models. Liu et al. designed CRISPR/Cas9 system delivered by engineered extracellular vesicles (EVs) to specifically knock out glutathione peroxidase 4 (GPX4), a key negative regulatory gene of ferroptosis. This system successfully induced ferroptosis in GBM cells, reducing the median effective dose (ED50) of radiotherapy by approximately 22%, and providing a novel and efficient combined treatment strategy for radiosensitization [31].

5.6 Pancreatic Ductal Adenocarcinoma (PDAC)

Pancreatic ductal adenocarcinoma (PDAC), termed "king of cancers" for its fibrotic tumor microenvironment (TME), early metastatic tendency, treatment resistance, and has a 5-year survival rate below 10%. Over 90% of cases carry KRAS mutations (mainly G12D and G12V), and synergistic inactivation of tumor suppressor genes such as TP53, CDKN2A, and SMAD4 collectively promotes the malignant progression of the disease. The physical barrier composed of cancer-associated fibroblasts (CAFs) and dense extracellular matrix results in less than 10% penetration of chemotherapeutic drugs into the core tumor region, and severely limits immune cell infiltration-posing a major bottleneck for current treatments.

In terms of targeted therapy strategies, direct intervention on the "undruggable" KRAS oncoprotein is the focus of PDAC. Jiang's team used the CRISPR/CasRx system (a variant of CRISPR/Cas13d system) to degrade the mRNA of mutant KRASG12D, achieving efficient silencing at the translational level. This reduced the KRAS-G12D protein level by 80–85% and achieved a 72% tumor growth inhibition rate in preclinical models [32]. This strategy of targeting RNA rather than DNA avoids permanent changes at the genomic level, providing a new therapeutic path for KRAS-driven PDAC.

CRISPR/Cas9 screening also identified potential target CDK7 for strengthening chemotherapy sensitivity in PDAC. Knockout and inhibition of it can boost gemcitabine and paclitaxel efficacy, overcoming chemoresistance via the STAT3-MCL1-CHK1 pathway.[33]

To overcome resistance, the Witz team performed CRISPR-mediated gene editing on olaparib-resistant PDAC cell lines, successfully knocking in pathogenic mutations of BRCA1/2. This increased the efficacy of PARP inhibitors by 4.3 times and reversed drug-resistant cells to a sensitive state [34]. This discovery not only verifies that the homologous recombination repair status is a key biomarker determining the efficacy of PARP inhibitors but also provides proof of concept for reversing drug resistance in clinical practice.

6. Current Challenges and Limitations

NSCLC

CRISPR/Cas9 technology faces three fundamental challenges in lung applications: carrier enrichment efficiency below 10% due to complex physiological barriers, and immunogenicity of Cas9 potentially triggering neutralizing antibody responses.

Future strategies should develop exosome-mimetic biomimetic carriers incorporating lung-targeting peptides like GE11, and use engineered humanized Cas9 variants (e.g., HiFi Cas9) to reduce immune risks.

TNBC

CRISPR/Cas9 faces three key challenges in TNBC: high genomic instability causes wide editing efficiency variation (15%--68%), especially in homologous recombination deficiency (HRD) subtypes; excessive collagen deposition limits vector penetration to <15%; and lack of reliable biomarkers (e.g., PD-L1 expression shows weak correlation) hinders patient stratification.

To address these, high-fidelity editors (Base/Prime Editors) should be adopted to minimize DSBs and genomic toxicity.

CRC

The gut microbiota and metabolites (e.g., butyrate) may modulate Wnt signaling via GPCR activation, potentially compromising editing efficacy or unintentionally accelerating tumor growth. The liver, as the main metastatic site of CRC, features a unique microenvironment-including sub-150 nm endothelial gaps and highly phagocytic Kupffer cells-that severely restricts vector delivery, leading to markedly reduced editing efficiency in metastases compared to primary tumors. Off-target editing in LGR5+ intestinal stem cells

could disrupt crypt homeostasis and mucosal integrity, raising long-term safety concerns.

HCC

The fibrotic microenvironment blocks vector delivery, reducing intratumoral efficiency. Tumor spatial heterogeneity causes uneven editing, risking resistant clones. HBV DNA integration may impair editing accuracy and cause genomic instability.

Future solutions include fibrotic-responsive smart vectors, spatial multi-omics for clonal targeting, and image-guided local delivery (e.g., ultrasound microbubbles) to improve efficacy and safety, enabling a leap toward clinical cure.

GBM

While intracranial delivery of CRISPR/Cas9 has advanced, efficiency remains limited. Tumor spatiotemporal heterogeneity promotes clonal escape, and potential off-target effects on neural cells require strict safety evaluation. Sustaining efficacy against developing clones presents further challenges.

Future development relies on interdisciplinary collaboration to create enhanced brain-targeted delivery systems-such as magnetic field guidance and focused ultrasound for BBB opening-identify vulnerabilities through functional screening, and integrate combination therapies with tumor-treating fields and immunotherapy.

PDAC

The highly fibrotic tumor microenvironment (TME) results in an intratumoral delivery efficiency of nanocarriers of less than 5%; clonal escape caused by tumor heterogeneity reaches a rate as high as 35%; and the potential off-target risk to normal pancreatic tissue still requires strict evaluation.

Future research should focus on developing stimuli-responsive delivery systems for the TME, employing high-fidelity genome editing tools to mitigate off-target risks, and use patient-derived organoid models to advance personalized precision therapeutics for PDAC.

7. Future Perspectives

Looking ahead, the next leap will come from integrating existing strategies: AI-designed nanocarriers responsive to stiffness, hypoxic pH and immune checkpoints will be refined in patient-derived organoid–on-chip platforms; single-cell multi-omics will dynamically prioritize tumor-specific targets, guiding use of ultra-high-fidelity editors (e.g., evoBE4max, twin-PE) to minimize off-target effects.

Integrating these data streams with adaptive clinical-trial frameworks-enabling dynamic treatment adjustment based on circulating tumor DNA feedback-will compress the traditional "bench-to-bedside" timeline from years to months. Automated GMP microfactories will further reduce production costs tenfold.

Collectively, converging dynamic targeting, precision editing, and agile clinical translation will establish CRISPR/Cas9 as a routine therapeutic modality for solid tumors.

8. Conclusion

Nowadays, achieving a crucial leap from "in vitro research" to "in vivo therapy", CRISPR/Cas9 gene editing technology demonstrates potential for targeted therapy, immune regulation, and combination treatment in solid tumors. By targeting oncogenes such as KRAS and EGFR, or regulating PD-1 and tumor microenvironment signaling, it enhances tumor sensitivity to conventional therapies and demonstrates encouraging anti-tumor efficacy in preclinical and early clinical studies.

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