

Application of CRISPR in the Treatment of Pancreatic Cancer

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Abstract:

As a highly invasive malignant tumor, pancreatic cancer (PC) has an increasing incidence rate and a long-term low 5-year survival rate. At present, CRISPR has been used in the research of PC to analyze the function of oncogenes, explore drug resistance mechanisms and develop treatment strategies. Most of them are in the stage of cell and animal experiments. However, there are still research gaps in tumor microenvironment penetration, low delivery efficiency in vivo, off target effects and treatment limitations caused by tumor heterogeneity. This article systematically reviews the CRISPR foundation, analyzes the pathogenesis of high-frequency mutant genes in PC, elaborates three major treatment strategies based on CRISPR, combs the progress of second-generation CRISPR, clarifies the current technical challenges, and proposes targeted vector development, high fidelity Cas protein application and other corresponding programs. This study provides a comprehensive reference for the clinical transformation of CRISPR in the field of PC, helping to break through the bottleneck of traditional treatment; However, the long-term safety of this technology in the human body still needs to be verified. Future research can rely on the third-generation CRISPR to optimize the targeted delivery system, explore the joint strategy with immunotherapy and chemotherapy, promote the technology to clinical implementation, and provide more accurate treatment schemes for PC patients.

Keywords: pancreatic cancer; CRISPR; Gene editing; tumor microenvironment.

1. Introduction

As a highly invasive malignant tumor, pancreatic cancer (PC) is a serious threat to global health. Its incidence rate is rising steadily, while the 5-year survival rate hovers at a long-term low level. As a rev-

olutionary breakthrough in the field of life science, CRISPR, with its advantages of simple operation, low cost, high editing efficiency and multiple gene editing, has emerged in basic research and clinical application, bringing new hope for tackling PC. This article will comprehensively review the application

of this technology in the treatment of PC, including basic research, treatment strategy exploration, delivery system development and clinical research progress, and objectively analyze the current challenges and future directions, in order to provide reference for promoting its clinical transformation.

PC is a malignant tumor with a high incidence rate worldwide, and its incidence rate is on the rise worldwide. According to the International Agency for Research on Cancer (IARC), PC ranks 12th in the world's most common malignant tumors, accounting for 2% of all cancer cases. More than 460000 people worldwide are diagnosed with PC every year, and the prognosis is poor. Regional differences in PC are large. incidence rate of PC is relatively high in developed countries such as North America, Europe and Australia, and relatively low in some countries in Asia and Africa. The incidence of PC may be related to unhealthy lifestyle, obesity, hyperglycemia, smoking, family history and genetic factors.

In recent years, the global incidence rate of PC is on the rise, and PC has become one of the most common causes of disease related deaths [1]. At present, the treatment of PC includes surgery, radiotherapy and drug therapy, but the treatment of PC still faces many challenges due to low early diagnosis rate, low drug response rate and drug resistance [2]

CRISPR/Cas9 is an efficient gene editing tool that uses sgRNA to guide Cas9 protein to cut DNA at specific locations, and achieves gene knockout insertion through cell repair mechanisms. Up to now, the application of CRISPR/Cas9 technology has been very mature. At present, this technology has been used for the functional analysis of oncogenes, the exploration of drug resistance mechanism, the mining of potential therapeutic targets and the development of precise treatment strategies for PC. Most of them are in the stage of cell and animal experiments. Some achievements have provided theoretical support for subsequent clinical transformation, but they still face challenges such as complex tumor microenvironment and low delivery efficiency in vivo [3].

This study focuses on the high-frequency mutation oncogene and microenvironment regulatory gene of PC, and uses CRISPR-Cas9 to knock out or repair the target gene to evaluate the impact on the proliferation and apoptosis of cancer cells; By combining animal models to verify the anti-tumor effect and safety, analyzing off target and normal tissue effects, exploring synergistic effects with immunotherapy and chemotherapy, providing support for precision therapy, and promoting its clinical translation.

2. Fundamentals of CRISPR

2.1 Types and Structure of CRISPR Cas System

The CRISPR Cas system is a natural immune system in prokaryotes used to resist invasion of exogenous genetic elements. According to different interference mechanisms, it can be divided into two categories, namely Class1 and Class2. Class1 consists of multiple proteins that form effector complexes, including types I, III, and IV. Taking type III as an example, it can be further divided into two subtypes, III-A and III-B, characterized by the presence of Cas10 protein. Cas10 assembles into a multi subunit effector complex with Cas5, multiple copies of Cas11, Cas7, and a crRNA. In addition, there is a novel III-E type, whose key protein Cas7-11 is a single stranded protein containing several Cas7 domains and one Cas11 domain, which can process pre crRNA into mature crRNA and specifically cleave single stranded target RNA [4].

Class2 functions by a single protein, including types II, V, and VI. Taking type II as an example, its representative is the CRISPR-Cas9 system. Cas9 is an endonuclease that contains the REC domain, RuvC domain, HNH domain, PI domain, etc. [5]. Among them, the REC domain connects to other domains, the HNH domain and the RuvC domain synergistically cleave DNA double strands, and the PI domain interacts with the PAM region of the target DNA, promoting cleavage specificity.

2.2 CRISPR Gene Editing Principle

The core principle of CRISPR originates from the adaptive immune system of prokaryotes, such as bacteria and archaea. In its natural state, when foreign pathogens such as bacteriophages invade, prokaryotes integrate characteristic DNA fragments of pathogens into their own CRISPR sequences, forming "immune memory". When the pathogen invades again, the CRISPR sequence will transcribe CRISPR RNA (crRNA) that is complementary to the pathogen's DNA and bind with transactivation crRNA (tracrRNA) to form a composite RNA, guiding Cas family endonucleases (such as Cas9) to recognize and cleave the invading foreign DNA, thereby clearing the pathogen [6]. The artificially modified CRISPR system fuses crRNA and tracrRNA into single stranded guide RNA (sgRNA), simplifying the operation. One end of sgRNA accurately recognizes target genomic DNA through complementary base pairing, while the other end binds to Cas enzyme to guide Cas enzyme localization to the target site. The two nuclease domains (HNH and RuvC) of Cas enzyme cleave two strands of DNA, producing double strand breaks (DSBs) [7]. Cells initiate DNA damage repair mechanisms: When repairing non homologous terminal junctions (NHEJ), the

broken ends are randomly connected, often resulting in base insertions or deletions, leading to gene knockout; If an exogenous DNA template is provided, homologous directed repair (HDR) will use the template as a blueprint to accurately repair breaks, thereby achieving gene insertion or mutation repair. Through this mechanism, CRISPR can efficiently and accurately edit target genes [8].

3. Pathogenesis and Related Genes of PC

The occurrence and development of pancreatic ductal adenocarcinoma (PDAC) are closely related to multiple high-frequency mutated genes, which disrupt normal cell signaling pathways, drive malignant phenotypes such as tumor proliferation, invasion, and drug resistance, and lead to heterogeneity within and between tumors. These genes are the core of understanding the pathological mechanism of PDAC [9].

RAS is the gene with the highest mutation rate in PDAC (about 85%), and normally acts as a “molecular switch” to regulate the cell proliferation signaling pathway. The mutation is mainly at codon 12 (such as G12D, G12V), which leads to sustained protein activation and is not regulated by upstream signals. It continuously drives pathways such as RAF/MEK/ERK, promotes abnormal proliferation of pancreatic ductal epithelial cells, inhibits apoptosis, induces tumor microenvironment (TME) fibrosis, enhances invasiveness and chemotherapy resistance, and is an early driving event of PDAC [10]. RAF, as an upstream effector molecule in the pathway, is recruited and activated by the continuously activated mutant RAS in PDAC, and then initiates downstream signaling through phosphorylation; MEK is a direct substrate of RAF, and upon activation, it can further phosphorylate and activate downstream ERK, playing a “signal relay” role in the pathway to ensure efficient transmission of oncogenic signals from mutated RAS to the effector end; ERK, as a key downstream effector kinase in the pathway, enters the nucleus after activation and directly drives abnormal proliferation and resistance to apoptosis of pancreatic ductal epithelial cells by regulating the expression of target genes such as Cyclin D1 (promoting cell cycle progression) and Bcl-2 (inhibiting apoptosis). At the same time, it also participates in inducing tumor microenvironment fibrosis by regulating the secretion of cytokines such as TGF - β and IL-6, ultimately enhancing the invasion ability and resistance to chemotherapy drugs of PDAC cells. It is the core signaling molecule driving the occurrence and development of PDAC by RAS mutations.

As a “guardian of the genome”, TP53 has mutations in

approximately 70% -80% of PDACs, mainly occurring in the DNA binding domain [11]. Mutation leads to the loss of p53 protein function, preventing the initiation of DNA damage repair or apoptosis programs, and even obtaining pro cancer activity, causing genomic instability and evasion of apoptosis in cancer cells. It also upregulates resistance genes to enhance chemotherapy resistance, which is closely related to the progression and poor prognosis of PDAC [12].

CDKN2A exhibits abnormalities (deletions or mutations) in 90% of PDACs, and its encoded p16INK4a can inhibit cell cycle progression. After the loss of function of this gene, the cell cycle is out of control, accelerating the progression of pancreatic intraepithelial neoplasia to invasive cancer, while enhancing the tumor’s resistance to radiotherapy, which is a key driver of early carcinogenesis in PDAC [13].

About 50% of PDACs in SMAD4 have mutations, which normally mediate the regulation of cell differentiation and apoptosis through transforming growth factor - β (TGF - β) signaling. Mutation leads to the loss of its function, causing the TGF - β signal to shift from “anti-cancer” to “pro cancerous”, enhancing epithelial mesenchymal transition (EMT), and improving tumor invasion and metastasis ability. Moreover, SMAD4 deficiency suggests poor prognosis and susceptibility to distant metastasis in patients.

4. CRISPR Strategy in the Treatment of PC

CRISPR in the treatment of PC, mainly through the precise targeting of key genes or microenvironments of tumor occurrence and development, design targeted strategies.

4.1 Directly Editing Oncogenes to Block Tumor Driving Signals

The principle of this strategy is that the oncogene with high frequency mutation in PC (such as KRAS G12D/V) will continuously activate the downstream proliferation signal pathway. CRISPR can specifically identify the mutant sequence through sgRNA, and guide Cas enzyme to cut the mutant gene to block the signal. The core argument of this strategy is that precise editing of mutated oncogenes can effectively cut off tumor driving signals and inhibit tumor growth. For example, the MIT team designed sgRNA for KRAS G12D and delivered CRISPR-Cas9 to PANC-1 cells and mutant mouse models through lipid nanoparticles. The results showed that the expression of oncogene KRAS G12D protein in cells decreased by 72%, and downstream extracellular signal regulated kinase phosphorylation decreased by 65%; In animal models,

tumor volume decreased by 58% and Ki-67 positivity rate decreased by 40%, confirming that this strategy can significantly block signaling pathways, inhibit proliferation, and validate the effectiveness of precise editing of oncogenes [14].

4.2 Restoring the Function of Tumor Suppressor Genes

Restoring the function of tumor suppressor gene can reconstruct the normal regulation of cells. Tumor suppressor genes such as TP53 and CDKN2A in PC often become inactivated due to mutation, resulting in disorder of cell regulation. CRISPR can restore its function by homologous directional repair and insertion of normal fragments [15]. The argument is that restoring the function of tumor suppressor genes can rebuild regulatory mechanisms and inhibit malignant phenotypes. Liu et al. inserted wild-type TP53 fragments into MIA PaCa-2 cells with TP53 inactivation using CRISPR. The results showed that the apoptosis rate increased from 12% to 45%, and G1 phase arrest increased by 30%; In the animal model, the tumor volume decreased by 62% and the expression of γ -H2AX increased by 2.3 times, indicating that restoring the function of tumor suppressor genes can initiate apoptosis and inhibit progression, supporting the feasibility of this strategy.

4.3 Modification of Tumor Microenvironment

The principle of transforming tumor microenvironment to enhance therapeutic sensitivity is that the fibrosis of PC microenvironment and immunosuppression form a therapeutic barrier. CRISPR can knock out related genes to reduce the barrier and remove the inhibition. The argument is that editing microenvironmental genes can enhance the sensitivity of traditional therapies [16]. Zhang et al. found that type I collagen encoded by COL1A1 is the core component of tumor matrix fibrosis. It is secreted in large quantities by alpha SMA myofibroblasts to form a dense physical barrier, which not only hinders the penetration of chemotherapy drugs such as gemcitabine into the tumor core, but also promotes tumor invasion and metastasis through matrix rigidity signals. Knocking out this gene can reduce fibrosis by 40% and directly relieve drug delivery barriers; PD-L1, as an immune checkpoint molecule, is highly expressed on the surface of tumor cells and tumor associated immune cells. By binding to PD-1 on the surface of CD8⁺ T cells, it initiates immune suppression signals, causing T cells to become disabled and escape attack. Knocking out PD-L1 can relieve this “immune brake”, increasing tumor infiltration of CD8⁺ T cells by 3.5 times and enhancing the anti-tumor activity of

PD-1 inhibitors. The combined editing of the two can simultaneously break down the physical barrier and immunosuppressive microenvironment of TME, creating conditions for chemotherapy drug penetration and immune cell killing, ultimately increasing the complete remission rate of combination therapy from 5% to 38%.

5. Existing Technology of CRISPR in the Treatment of PC

5.1 Second Generation CRISPR

In order to overcome the limitations of CRISPR relying on DSB and other technologies, second-generation CRISPR has been developed. The second-generation CRISPR mainly includes base editors (BE) and prime editors (PE), which aim to improve the accuracy and diversity of gene editing while reducing potential side effects such as genetic toxicity. BE utilizes partially inactivated Cas protein (dead Cas9, dCas) to fuse with specific deaminase and achieve single nucleotide conversion through base modification; PE uses Cas9 endonucleases (nCas) fused with reverse transcriptase and specially designed primary editing guide RNAs (pegRNAs) to achieve more flexible genome editing, including inducing deletions, insertions, and various base substitutions of small nucleotide sequences. In addition, CRISPR can also be used in the fields of RNA editing and epigenetic regulation, such as CRISPR-Cas13 for targeted editing of RNA, and dCas9 fusion protein with epigenetic regulatory factors (methyltransferase, demethylase, histone acetyltransferase, etc.) for epigenetic regulation. The third generation CRISPR is on the rise. These advances include achieving precise insertion of ultra large DNA fragments such as CRISPR related transposition systems; developing novel Cas proteins such as Cas12f to construct smaller volume gene editing tools; utilizing light sensitive or drug inducible Cas proteins for spatially and temporally controllable gene editing; and developing multi gene editing systems capable of simultaneously targeting multiple gene loci. These developments are expected to enable more precise and complex genome manipulation, opening new prospects for studying complex genetic networks in oncology [5, 7].

5.2 Challenges Faced

The current challenges include insufficient delivery efficiency and targeting due to PC's dense fibrotic stroma and immunosuppressive microenvironment, making CRISPR modules (Cas protein and gRNA) difficult to penetrate and accurately reach tumor cells while being easily ingested by normal tissues, leading to off-target effects and toxic

side effects; limitations in editing efficiency and repair mechanisms as PC cells are mostly slow-dividing solid tumors where precision editing efficiency relying on HDR is extremely low while NHEJ is prone to trigger random mutations, affecting therapeutic outcomes; significant tumor heterogeneity and drug resistance since PC exhibits a high degree of genetic heterogeneity, making single-target editing inadequate to cover all tumor cells and frequently leading to recurrence of drug resistance in residual cells, while the editing process may activate compensatory signaling pathways (such as bypass activation in KRAS mutant cells), thereby weakening treatment efficacy; and immunogenicity and safety risks wherein exogenous Cas proteins (such as SpCas9) may provoke immune responses in the body, resulting in clearance of the editing system, and off-target editing could disrupt normal genes (such as tumor suppressor genes) and increase the risk of carcinogenesis.

5.3 Response Strategies

Current strategies focus on optimizing the delivery system by developing targeted vectors such as lipid nanoparticles (LNP) or viral vectors modified with PC specific antigens like MUC1 to enhance tumor cell recognition and penetration. This is complemented by modulating the tumor microenvironment using agents such as hyaluronidase to degrade stromal fibrosis and improve CRISPR delivery efficiency. To enhance editing accuracy and efficiency, efforts include engineering novel Cas proteins like high fidelity Cas9 (eSpCas9) to minimize off target effects and employing Cas12a (Cpf1) for greater editing flexibility, as well as utilizing BE or PE to enable precise edits without DSBs and avoid the unpredictability of NHEJ. Additionally, regulating the cell cycle using pharmacological agents can induce PC cells into a more editable state and improve HDR mediated repair efficiency. To address tumor heterogeneity, multi target combination editing strategies are being pursued, simultaneously targeting key genes such as KRAS and TP53 or combining with immune checkpoint genes like PD-L1 to cover diverse tumor subpopulations. These approaches are further supported by integration with conventional therapies; for instance, CRISPR mediated knockdown of resistance genes like ABCG2 is combined with chemotherapy such as gemcitabine or immunotherapy such as PD-1 inhibitors to reduce recurrence risks. To mitigate immunogenicity and safety concerns, low immunogenicity Cas proteins such as humanized variants or engineered Cas9 are deployed to minimize immune reactions, while mRNA or protein based delivery formats are used to shorten in vivo exposure to exogenous components. Finally, high throughput off target

detection methods, including whole genome sequencing and optimized gRNA design, are employed to identify and minimize potential off target sites and enhance specificity. CRISPR gene editing organ like technology is completely changing the way of research and treatment of PC. Although we still face some technical challenges mentioned above, with the continuous optimization of methods and the development of new technologies, this joint strategy is expected to play an increasingly important role in basic research and clinical transformation of PC. In the future, CRISPR gene editing organ models are expected to make breakthroughs in basic research, drug development, precision medicine and gene therapy of PC, bringing new hope to PC patients.

6. Conclusion

This article systematically reviewed the core content of CRISPR gene editing technology in the treatment of PC, covering the technical basis, the carcinogenic mechanism of high-frequency mutation genes related to PC, and the three major treatment strategies based on CRISPR. At the same time, it reviewed the application progress of second-generation CRISPR, analyzed the current challenges such as low in vivo delivery efficiency, off target effect, and tumor heterogeneity, and put forward solutions such as targeted vector development, high fidelity Cas protein application, and multi target collaborative editing. This review provides a clear technical framework for the treatment of PC, echoes the high incidence rate, low survival rate of PC and the challenges of traditional treatment in the introduction, and clarifies the core value of CRISPR in accurately targeting key tumor genes and microenvironment. The target verification, strategy optimization and safety improvement path it combs can directly provide reference for subsequent basic research and clinical transformation, and help break through the dilemma of PC treatment. Current research is mostly in the stage of cell and animal experiments, and the long-term safety of CRISPR systems in humans and their adaptability to complex tumor microenvironments still need further validation. Future research can rely on the third-generation CRISPR, combined with organ like models, to develop more efficient targeted delivery systems, explore the combined strategy of CRISPR, immunotherapy and chemotherapy, promote the transformation of technology to clinical practice, and ultimately provide more accurate and safe treatment schemes for PC patients.

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