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Advances in CRISPR-Cas9 Technology for Tumor Therapy

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Abstract:

Genome editing technology is an emerging toolbox that can specifically target genes. At present, the existing gene editing technologies include ZFN, TALEN, and CRISPR system editing technology with clusters of regularly spaced short palindromic repeats. However, ZFN and TALEN are no longer the preferred tools for gene editing due to some of their problems, and their complex protein design, high cost, and high difficulty are the main reasons that limit their use. The CRISPR system, on the other hand, is able to achieve specific binding by base complementary pairing of sgRNA to the target sequence. Traditional cancer treatment methods have limited efficacy and high recurrence rates, and the disease can be fundamentally treated by inactivating oncogenes or activating tumor suppressor genes through gene editing technology and then changing downstream signaling pathways for cancer treatment. With CRISPR-Cas9 technology, it is possible to have gene knockouts, insertions, and mutations. Here, we will investigate and explore the feasibility of the CRISPR system for tumor treatment, including the following three topics: oncogene knockout, augmentation of tumor suppressor gene expression, and enhancement of engineered T cell viability for effective tumor Through these methods, the CRISPR system is of great help and promising tumor therapy.

Keywords: Gene editing;CRISPR-Cas9;Tumor Therapy.

1. Introduction

Gene editing permits the change of specific genes thereby altering their genetic information and phenotypic characteristics. Currently, reported gene editing technologies include zinc finger nucleases (ZFNs)[1], transcription activator-like effector nucleases (TALENs)[2], and the emerging clustered regularly interspaced short palindromic repeats (CRISPR) /CRISPR-associated proteins (Cas) system[3]. ZFN can realize efficient site-directed gene modification, which consists of two parts: a zinc finger protein (ZFP) domain and a cleavage domain (FokI). The ZFP domain is a tandem structure of 3~4 zinc finger (ZF), each ZF contains about 30 amino acids and is immobilized by 1 zinc ion and each of them can identify and combine with one triplet base which is also known as codon. One zinc finger can identify three to four codons. The two FokI cleavage domains form the active form of the dimer, forming a double-strand break (DSB)[4]. Another type of gene editing technology is TALENs. TALEN consists of TALE and Fok1 (Flavobacterium okeanokoites)[5]. TALE comprises of three parts: N-terminal transport signal, central DNA-specific recognition binding domain, C-terminal nuclear localization signal, and transcriptional activation domain. The DNA-specific recognition binding domain consists of a string of repetitive units, and amino acids at position12 and 13 of each repeat are highly variable, which are called repeat variable residues (RVD), and can correspond to A, T, G, and C bases[6]. NI specifically recognizes A, HD specifically recognizes C, NG recognizes T, NH specifically recognizes G, NN specifically recognizes G or A, and NS can recognize any of A, T, G, or C. However, since both ZFP and TALEN recognize specific sites through protein-DNA interactions, complex protein design, expensive cost, and high difficulty still limit the wide application of these. The CRISPR-Cas system can modify the genome, and target DNA sequence recognition depends on a guide RNA (gRNA)[7]. Compared to ZFN and TALEN, the CRISPR-Cas system achieves specific recognition by the base complementary pairing of DNA and RNA. Due to its simplicity, low cost, scale, high throughput, high editing efficiency, and low experimental requirements, the CRISPR-Cas system is rapidly applied to various research fields.

Tab.1 The differences of ZFN, TELEN and CRISPR-Cas.

Category	ZFN	TALEN	CRISPR-Cas
Source	Eukaryotic transcription regulators	Xanthomonas	Prokaryotes
Recognize patterns	Protein-DNA	Protein-DNA	RNA-RNADNA
DNA recognition domain	ZFA protein tandem (generally 3~4, each ZFA recognizes and binds 1 specific triplet base)	TAL protein tandem composition (generally about 20, each TAL recognizes and binds a corresponding base)	Guide RNA
Endonucleases	Fokich aggregates	Folky dimer	Cass protease
Identify the length	(3~6)x3x 2bp	(12~20)x 2 bp	About 20 bp near the PAM sequence
Cytotoxicity	big	relative small	small
Easy to build	hard	relative easy	easy
Type of cut	Double-strand fracture, single-strand notching	Double-strand fracture, single-strand notching	Double-strand fracture, single-strand notching
The ease of delivery in vivo	relative easy	hard	secondary
Advantage	The platform is mature and the cutting efficiency is high	High cutting efficiency, low off-target rate, large-scale build, high-throughput assembly	The cutting efficiency is the highest, multiple sites can be targeted at the same time, the operation is simple, the time is short, and the cost is low
Disadvantage	The operation is complex, costly, off-target efficient, and cytotoxic	Costly, cytotoxic, and cumbersome module assembly	There is a certain off-target effect, and NHEJ will randomly produce toxicity

2. Mechanism and classification

The CRISPR-Cas system is an acquired immune mechanism of some bacteria and archaea[8]. CRISPR is constituted of a leader sequence, multiple short, conserved regions of repeats, and many spacers.[9] The leader sequence is located upstream, repeats are highly conservative, and spacers are the traces left after the invasion of phages or plasmids, thus giving the cell access to the corresponding phage and plasmid immune defenses[10].

The CRISPR-Cas system contains three stages: adaptation - Ingestion of foreign genetic material: After the invasion of foreign genetic material, the CRISPR-Cas system recognizes the PAM sequence of the foreign gene, and obtains part of the foreign fragment from the vicinity of the PAM sequence to form a spacer sequence, which is integrated between the CRISPR repeats from the 5′ end, so that it has a "memory" that can specifically destroy the invading genetic material in the next infection, most of the known CRISPR-Cas The system requires the participation of the Cas1-Cas2 complex in this process[11]; Expression – crRNA expression and maturation: CRISPR regions are first transcribed into pre-crRNA, then cleaved into mature crRNAs containing 1 spacer sequence and some repeats, which bind directly or further to Cas proteins into "effector" or "interfering" complexes with specific endonuclease activity[12]; Interference – Cutting of exogenous genetic material: The complex is scanned along the exogenous genetic material under the guidance of crRNA, and when there is a crRNA matching region near the PAM sequence, the effector complex is cleaved to decay DNA molecules, and the expression of exogenous nucleic acids is silenced[13].

There are many types of CRISPR-Cas systems, which contain two broad categories based on the effector proteins[14]. The first major class of effector proteins of the CRISPR-Cas system is composed of multi-subunit effector complexes containing 4-7 Cas proteins[15]. Class 2

CRISPR-Cas systems contain only a single multidomain effector protein[16]. The two major categories of systems are further subdivided into 6 types, with the first category containing types I, III, and IV, and the second category containing types II, V, and VI[17]. Depending on the characteristic protein, types I, II, and III can be distinguished. Because of the simple structure of the effector complex, type II CRISPR-Cas systems have been modified to be genome editing toolboxes.

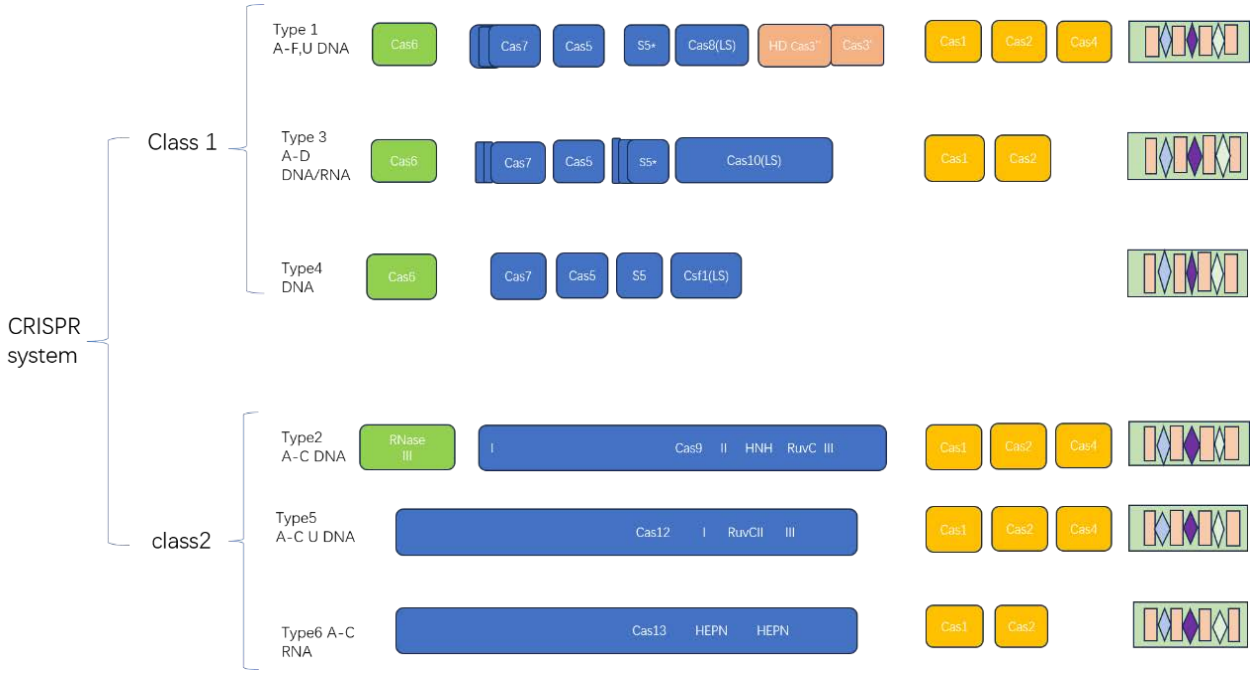

Fig.1 The classification of the CRISPR system.

2.1 CRISPR-Cas9 system

Cas9 protein includes HNH and RUVC nuclease domain. HNH domains are used to cleave the complementary DNA strands and the RUVC nuclease is used to cleave the non-complementary strands[18]. SgRNA is constituted of CRISPR RNA (crRNA) and transactivation RNA (tracrRNA)[19] .In nature, there are approximately 90% of archaea and 40% of bacteria immune from foreign viruses or phages via the CRISPR-Cas systems that exist on the genomes and plasmids[20]. When viruses or phages first inject the genome into the bacteria, the Cas protein will integrate a small segment of the sequence on the exogenous genome into the 5' end of the CRISPR sequence, forming an "immune memory" of the foreign genome. When the bacteria are invaded again, the CRISPR sequences which contain information on exogenous genetic material will transcript and form sgRNA. Then the sgRNA combines with the Cas protein to cleave specific foreign DNA. After the specific foreign DNA was cleaved, bacteria successfully immunized against foreign viruses or phages[21].

At the time of gene editing, Cas9 first forms a complex with sgRNA, it is then up to Cas9 to identify the protospacer adjacent motif also known as PAM. After that, the sgRNA binds specifically to the target sequences. Next, Cas9 cleaves double-stranded DNA, making the double-strand DNA breaks(DSB). As a result, the broken DNA will repair itself by non-homologous end joining(NHEJ)or homologous recombination (HR) [22].

2.2 Gene editing tools

Compared to HR and NHEJ, the single-base editing technique is more efficient, and versatile, it does not depend on the cell cycle and has a low lethality. Single-base editing techniques are performed by inactivating dCas9 (H840A for the HNH domain and D10A for the RuvC domain) or Cas9 nickase (Cas9n; D10A or H840A), which forms a fusion protein with cytidine deaminase or adenosine deaminase, and performs single-base editing within the target site editing window without generating DSB[23]. Existing single-base editing techniques include cytidine base editors (CBEs) that change cytosine (C) to thymine (T) and adenine base editors (ABEs) that change adenine(A) to guanine(G). Two systems have been developed based on the single-base editing, CBE and ABE[24]. Komor et al. developed a CBE system fused with murine cytosine deaminase apolipoprotein B mRNA-editing enzyme catalytic polypeptide (APOBEC1), which uses APOBEC1 to deaminate C to uracil (U), which then recognizes U as T to achieve C→T conversion. The first-generation base ed-

iting BE1 (APOBEC1-XTEN-dCas9) achieves an editing efficiency of 25%-40% in vitro with an editing window of positions 4-8 of the sgRNA (position 1 is the first nucleotide of the distal sgRNA of the PAM, and the PAM is located at positions 21-23. BE1 is greatly less efficient in vivo because the resulting intermediate, U, is uracil-based DNA N-glycosylase (uracil DNA N-glycosylase (UNG) [25] recognizes and excises, activating the base excision repair (BER) pathway to restore the intermediate product U•G to its original sequence C•G. Researchers developed BE2 by fusing a uracil DNA glycosylase inhibitor (UGI) based on BE1 and then combined with the endogenous mechanism of the cell, replacing dCas9 in BE2 with nCas9 stimulates the intracellular mismatch repair (MMR) pathway to develop BE3 It can greatly improve editing efficiency and product purity. Using a strategy similar to CBE, Gaudeli et al. reported in 2017 that the ABE system utilizes evolved TadA* deoxyadenosine deaminase to convert adenine (A) within the R-loop to inosine (I), which is recognized by DNA polymerase as G, thereby achieving A→G conversion. Since adenine deaminase is known to be unable to deaminate A with DNA as a substrate, the researchers performed directed evolution of E. coli TadA and successfully screened for ecTadA* with 14 mutation sites, and developed an ABE7.10 system (ecTadA-ecTadA*-nCas9) that can directly act on ssDNA[26].

Prime editor (PE) is another new method of gene editing, in which a protease is fused with Cas9n (H840A) and reverse transcriptase, and the engineered guide RNA (pegRNA) is bound to a primer binding site (PBS) and RT template sequence at the 3'end. The protease is guided to locate and cleave the non-complementary strand of dsDNA, PBS is paired with the complementary sequence recognition before the cleavage site, and the reverse transcriptase uses the transcription template sequence as a template for reverse transcription, polymerizing the target sequence directly onto the nicked DNA strand[27]. In this process, the fusion protein assumes the dual function of cleavage of the target site non-target strand and reverse transcription, while the pegRNA both guides the PE to recognize the target site and contains the information required for editing. Through these two components, the PE system achieves a series of processes such as recognition, cleavage, primer sequence binding for initiating reverse transcription, reverse transcription, etc., and directly reverse transcribes the required DNA information to the break at the target site[28].

The crystal structure of dCas9-sgRNA showed that the loop structure of sgRNA does not bind to Cas9 protein, and changing the original loop to an aptamer that can interact with transcriptional regulatory protein can further enhance the transcriptional regulation of Cas9 protein, and perform transcriptional activation (CRISPRa) or inhibition (CRISPRi) of gene expression at the gene level without changing the gene level[29]. CRISPRi and CRISPRa regulate gene expression rely on the continuous expression of Cas9 fusion proteins, and are not suitable for cell therapy. Compared to HR and NHEJ, these techniques are highly efficient, and versatile, do not depend on the cell cycle, and have a low lethality[30].

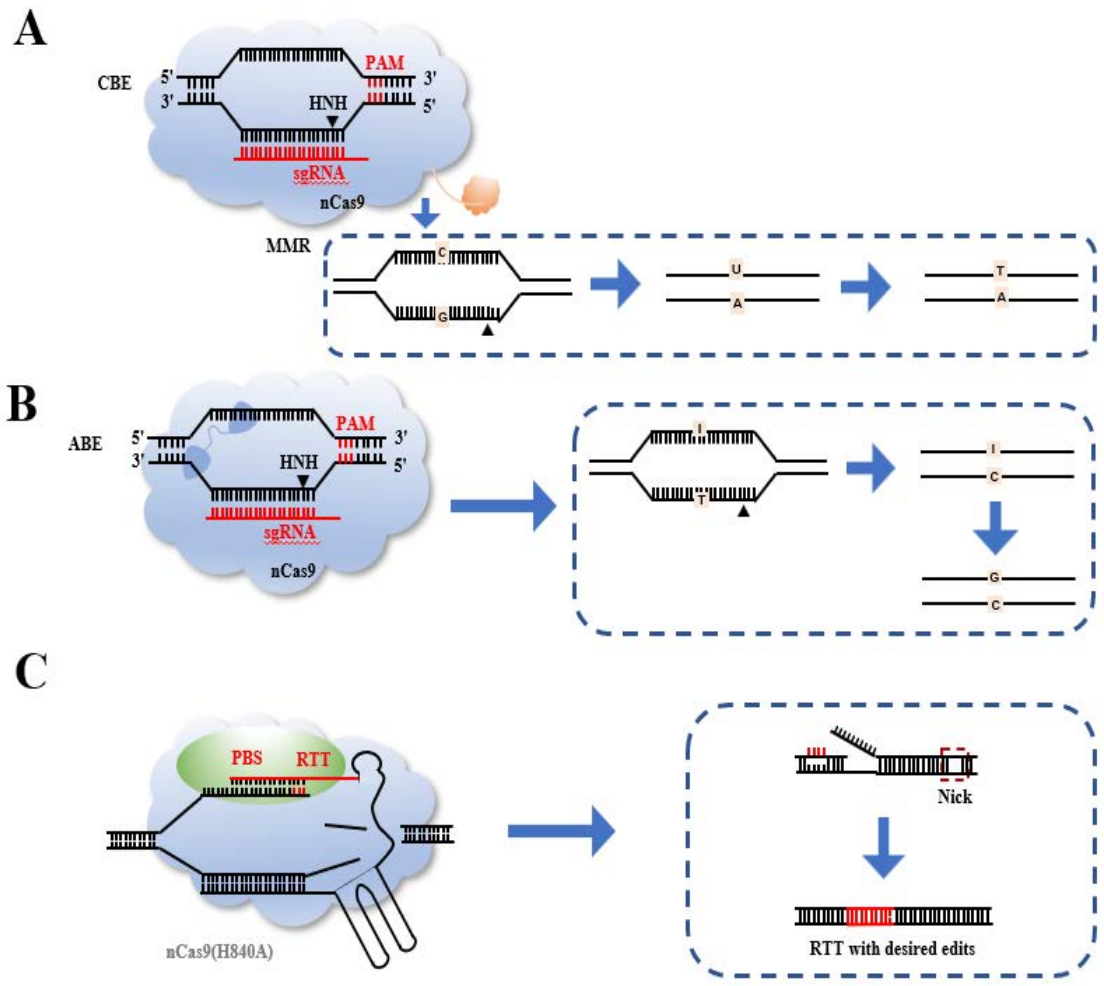

Fig.2 Other gene editing tools A: the mechanism of CBE B: the mechanism of ABE C: the mechanism of PE.

3. The application of CRISPR-Cas9 technology

Tumorigenesis is formed by the accumulation of somatic mutations, and different mutation genes and mutation sites depend on the type of cancer. Traditional cancer treatment methods have limited efficacy and high recurrence, and the disease can be fundamentally treated by inactivating oncogenes or activating tumor suppressor genes through gene editing technology, and then changing downstream signaling pathways for cancer treatment.

3.1 Enhance the expression of tumor suppressor genes to inhibit tumor progression

Tumor suppressor genes can promote cell differentiation, and inhibit excessive proliferation and migration of cells, and tumor suppressor genes will cause tumors once they are inactivated and mutated. CRISPR-Cas9 gene editing technology can integrate suppressor genes into tumor cells, and specifically repair mutations in suppressor genes, achieving the restoration and enhancement of the activity and function of suppressor genes, and is important to repress the development of tumors.

The first method is about using CRISPR-Cas9 to introduce tumor suppressor genes to inhibit tumor progression. When the mRNA of the tumor suppressor gene PTEN selects the CUG start codon instead of the ATG start codon during translation, the translated protein will have 173 amino acids at the N-terminus, called PTENlong. PTEN-long not only kills tumors like traditional tumor suppressor proteins but also can cross cell membranes and secrete, which has potential application value in tumor treatment. Cas9 is combined with single-stranded templates to target the CTG start codon of PTEN-long to enhance the expression of PTEN-long. After the CUG codon was transformed into the AUG codon, the CUG codon significantly increased the PTEN-long translation compared with the CUG codon of the original PTEN mRNA. Compared with the wild type, PTEN-long overexpressing

U87 cells significantly reduced Akt phosphorylation levels and inhibited the proliferation of glioma U87 cells. In summary, CRISPR-Cas9-driven PTEN-long can enter and exit cells and inhibit the proliferation of neighboring cells, suggesting that CRISPR-Cas9-generated PTEN-long may be an alternative strategy for PTEN gene therapy[31].

The second method is about using CRISPR-Cas9 to correct deleterious mutations to inhibit tumor progression. Traditional chemotherapy and radiotherapy methods do tremendous damage to normal cells while killing tumor cells. To solve this problem, the researchers established a tunable dual promoter logic pathway to modulate the expression of CRISPR-Cas9. The specific highly expressed promoter hTERT in tumor cells was fused with sgRNA, and the specific highly expressed promoter hUPII in bladder tissue was fused with the Cas9-expressing gene. The CRISPR-Cas9 system can be activated when both promoters are highly expressed in a cell line to edit genes downstream of it. BAX can promote apoptosis, p21 inhibits cell cycle progression, and high expression of E-cadherin negatively regulates tumor metastasis. The expression of the above three genes was controlled by lac-inducible promoters, and the CRISPR-Cas9 system regulated the expression of LacI. When CRISPR-Cas9 is expressed, it inhibits the production of LacI, so that the above three genes can be expressed, inhibiting tumor progression [32].

3.2 Targeted knockout oncogene for tumor therapy

The activation of oncogenes, which causes cells to divide indefinitely and continue to proliferate, is an important cause of tumor chemotherapy resistance. Oncogenes include cellular oncogenes and viral oncogenes, and many tumors such as liver cancer, cervical cancer, and nasopharyngeal cancer are caused by viruses. Targeted knockout of virus-associated oncogenes is critical for the treatment of tumors. Human blood virus(HBV) infection leads to liver cancer[33,34]. In 2016, Song et al.[34] designed 6 sgRNAs against ORF preS1/preS2/S, constructed the sgRNAs into CRISPR-Cas9 expression vectors, and finally obtained HBs Ag knockout hepatocellular carcinoma strains. This study showed that most of the sgRNAs significantly inhibited the production of HBs Ag [34].

In addition to viral oncogenes, oncogenic fusion genes are also involved in the development of many tumors. A fusion gene is a gene that has been originally isolated and whose sequences are fused in whole or in part as a result of a mutation to produce a new or abnormal gene [35]. Fusion genes in cancer cells usually express abnormal proteins, while normal cells do not have fusion genes, so targeting fusion genes is a good anti-cancer option. In 2017, Chen et al. [35] used CRISPR-Cas9 in prostate cancer and break sites targeting transmembrane protein135 (TMEM135)- coiled-coil domain-containing 67 (CCDC67) and alpha-mannosidase 2 (MAN2A1)-FER fusion genes in hepatocellular carcinoma cells; the herpes simplex virus type 1 phosphorylates thymidine (HSV1-TK) gene is then inserted cis-at the break site, and both ends of the break are linked simultaneously. Phosphorylation of HSV1-TK is stimulated with ganciclovir. Cytotoxicants are produced, causing apoptosis of these cancer cells [35]. When these CRISPR-Cas9-edited liver and prostate cancer cells were seeded into mice, tumors formed in the experimental group were significantly reduced in size and had little to no metastasis compared to the control group [35].

Fusion genes (FOs) are specific to cancer cells, and targeting fusion genes with gene therapy is highly specific, this approach may significantly reduce side effects when translated into clinical applications. Martinez-Lage et al. [36] used the CRISPR-Cas9 system to design a simple, effective, non-patient-specific gene editing method based on targeting two intron sequences to induce cancer cell-specific gene deletions, thereby eliminating key protein domain, or altering the FO reading frame. Ewing sarcoma is characterized by the fusion of EWSR1 with ETS protein, which causes tumorigenesis. The researchers devised a method to specifically delete the fusion gene, which induced the deletion only in FO-containing cells without affecting the expression of other genes, and the experimental results showed that the CRISPR-Cas9 element targeting the FO was effective in suppressing tumor growth.

Zhen et al. [37] used CRISPR-Cas9 technology to knock out E6 and E7 protein-related genes that promote the development and maintenance of cervical cancer malignancies. They exposed CRISPR-Cas9 cells targeting E6 and E7 to CDDP, which can significantly inhibit the growth of cells in vitro, indicating that CRISPR-Cas9 targeting E6+E7 can be used as a sensitizer for CDDP.

3.3 CRISPR-Cas9 attacks tumors by increasing engineered T-cell viability

Immune cells can immune surveillance, recognition, and killing of malignant tumors, and the CRISPR-Cas9 system to produce genetically engineered immune cells to destroy cancer cells more specifically and efficiently has gradually developed into a popular field of modern cancer treatment[38].

3.3.1 Tumor killing by knocking out immune checkpoint-engineered T cells

Lu et al. deleted the PD-1 gene of T cells by CRISPR-Cas9. The edited T cells are expanded and then reinjected into the bloodstream of lung cancer patients. High

expression of PD-1 as an immune checkpoint inhibits T cells from starting an immune response. Many lung cancer patients have high expression of PD-1 legend 1 (PD-L 1), which activates PD-1 and prevents T cells from killing tumors [39].

Dong et al[40]. found that the RNA helicase DHX37 (DEAH-box RNA helicase) is also an important immune checkpoint. DHX37 inhibits T cell activation, cytokine production, and cytotoxicity. Mechanistic studies have found that DHX37 interacts with the transcription factor p65 and the binding protein PDCD11 (programmed cell death 11) of the NF-κB pathway, thereby enhancing the activity of NF-κB. In CD8-positive T cells, knockdown of DHX37 enhances the effectiveness of adoptive immunotherapy in triple-negative breast cancer. In addition, Ye et al found that knockout of PDIA3 (protein disulfide isomerase family A, member 3) in specific types of T cells can enhance anticancer properties against human glioblastoma.

3.3.2 Modify the ability of chimeric antigen receptor T-cell (CAR-T) to recognize antigens

CAR-T cell immunotherapy is a type of adoptive cell immunotherapy for tumors[41]. CARs typically include extracellular tumor antigen recognition regions and intracellular signaling activation regions. T lymphocytes express chimeric antigen receptors that recognize tumor cells simultaneously activate T cells, and ultimately kill tumor cells in a non-major histocompatibility complex (MHC) manner. The advantage of CAR-T is that it can recognize tumor proteins and lipid antigens independently of MHC, without going through antigen-presenting cells, and has now developed into the fourth generation. It is mainly used for the treatment of hematological tumors such as leukemia and B-cell non-Hodgkin lymphoma, and in recent years, it has also been used to treat solid tumors[42-44].

Traditional CAR-T therapy has certain drawbacks, such as the limited number of T cell treatments, the long time of genetic engineering to modify T cells, and the high difficulty and cost. [45] CRISPR-Cas9 technology allows for easier modification of CARs or TCRs, thereby increasing the effectiveness and efficacy of T-cell-based therapies for tumors. [46]. For example, CRISPR-Cas9 was used to "site-site" integrate a CAR gene fragment targeting CD19 into TRAC, the encoding gene for TCRα, so that CAR expression is regulated by TRAC, enhancing the therapeutic effect of CAR-T cells in the NSG (NOD-scidgamma) mouse model of acute lymphoblastic leukemia (ALL)[47]. In addition, the knockdown of the CD33 gene in hematopoietic stem cells using CRISPR-Cas9 enables anti-CD33-CAR-T cells to specifically eliminate acute myeloid leukemia (AML) tumor cells without affecting normal myeloid cells [48].CRISPR-Cas9-modified CAR-T cells such as Stadtmauer[49] can can stably exert their killing function. First, the researchers collected T cells in the blood of the patients and eliminated two genes encoding the TCR chain, TCRα (TRAC) and TCRβ (TRBC), to reduce the mismatch of TCR. At the same time, the synthetic cancer-specific TCR transgene NY-ESO-1 (New York) was enhanced esophageal squamous cell carcinoma antigen 1); In addition, PDCD1, the gene encoding the immune checkpoint PD-1, was also deleted to improve the killing ability of T cells. The study showed that the edited T cells survived for up to 9 months in the human body, compared to the original CAR-T cells, which survived less than 7 days in vivo.

3.4 Application of CRISPR-Cas9 in antibody-targeted therapy

Schumacher et al. (2015) and Verdegaal et al. (2016) found that tumor cell surface antigens are different from normal cells, and their specific antigens become targets for immunotherapy, and antibodies against these targets can kill tumor cells to achieve anti-tumor effects [50, 51]. CRISPR-Cas9 gene editing technology enables the identification of neoantigens on the surface of tumor cells, providing a target for targeted antibody therapies [52]. Screening genes required for cancer cell fitness is the key to finding targeted drugs. Behan et al. [53] used CRISPR-Cas9 to destroy genes in more than 300 cancer cell lines of 30 cancers, screened genes required for cancer cell survival, and developed a prioritization system to prioritize genes that are most promising for the development of target drugs, which advanced the development of targeted therapies. CRISPR-Cas9 edits specific genes of antibodies, regulates antibody class switching, and diversifies antibodies, providing an effective strategy for the preparation of tumor-targeted antibodies [54]. Liu et al. [55] found that CRISPR-Cas9 alone or in combination with sunitinib to eliminate overexpressed EGFR may be a novel therapeutic option for renal cell carcinoma. Lee et al.[56] screened erlotinib susceptibility genes by inducing knockout mutations in erlotinib-resistant human lung cancer cells (NCI-H820) through a CRISPR-Cas9 sgRNA library.

4. Conclusion

The heterogeneity of tumors includes heterogeneity between and within tumors. Different parts of the same patient's tumor may have different gene mutations and expression profiles, which poses a great challenge to traditional targeted therapy. The CRISPR-Cas9 system is a revolutionary cancer treatment that breaks through the limitations of traditional methods of diagnosing and treating tumors. CRISPR-Cas9 holds the promise of offering

effective treatment for highly heterogeneous tumors by editing multiple genes simultaneously.

However, there are still some problems that need to be solved urgently: (1) The irreversible consequences caused by off-target are a major obstacle that restricts the CRISPR/Cas system from basic research to clinical practice [57,58], in which the PAM sequence, structure, length, and target location of sgRNA will affect the targeting efficiency, and the targeting efficiency can be modified by optimizing the PAM [59], appropriately designing and modifying the sgRNA sequence [60], and changing the Cas9 protein domain [61] (2) The efficiency of the delivery CRISPR system determines the efficiency of gene editing, and viral vectors are currently the most widely used, and some safer and more effective delivery tools such as polymers, liposomes, and nanomaterials are still needed [62,63], or Cas9 isoforms with a wider range of targets, higher specificity, and smaller molecules [64]; (3) Recently, more and more CRISPR/Cas systems are toxic to embryonic development and cell growth [65,66], and the causes of these should be explored and safer Cas tools should be developed; Protein and sgRNA can effectively address this problem by reversible regulation of gene expression at the transcriptional level. In the biomedical field, the CRISPR-Cas9 system develops new natural medicines, designs immune cells to treat tumors, builds gene circuits using modular functional elements, screens drug-resistance genes, develops new kit diagnostic systems, and develops vaccines. Single-cell multi-omic sequencing technology was used to study the genetic lineage and development microenvironment of tumors [67,68]. With the deepening of research, CRISPR-Cas9 will play a greater role in basic research and clinical application of tumors.

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