Development and Applications of CRISPR/Cas system in cancer treatment

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Abstract. Cancer has been the most severe fatal illness that has bothered people for centuries, it's caused by the combined action of various factors such as environmental factors, genetic factors, and the immune system of the body, and their pathogenesis is complex, and the specific mechanism cannot be fully elucidated. Humans search for cure methods to heal cancer. The main treatment for malignant tumors is chemotherapy, surgery, and radiotherapy. For those who are intolerant to chemotherapy and radiotherapy or those who miss their early stage and have lost the opportunity for surgery, there is still no specific and effective therapy for patients. This leads to poor prognosis for malignant tumors of patients and a low cure rate. As people continuously explore new solutions, an experimental tool is emerging and providing hopes of curing. This tool is CRISPR/Cas system. By editing cells' DNA that results in changes in cells' expression, the CRISPR/CAR system may someday be able to diminish tumors without side effects. CAR-T therapy is one of the experimental therapies that altered T-cells to diminish infected cells. Currently, CAR-T cells are still encountering challenges and obstacles. After altering CAR-T cells by applying CRISPR/Cas system, the efficiency in inhibiting cancer cells increases. Applying the Cas system in developing CAR-T therapy is possible that this therapy can treat cancer patients on a large scale, have less or no side effects and increase survival rates of cancer.

Keywords: CRISPR/Cas system, CAR-T therapy, UCAR-T, cancer.

1. Introduction

In 2022, 1918030 newly diagnosed cancer cases and 609360 cancer death occurred in the United States [1]. In 2023, as a major disease and second leading death factor in the United States, cancer has been estimated to cause 609,820 people's death which corresponds to 1670 deaths per day [2]. Some strategies for cancer treatment involve extracellular vesicles, nanomedicine, and gene therapy. Extracellular vesicles (EV) used to be an essential part of the identification of diagnosing cancer. It can be exploited and isolated to serve as anti-tumor vaccines for cancer therapy. The limitation of EV contains isolation, storage, standard protocols, and quantification for drug loading. Conventional chemotherapeutic drugs are now carried by nanoparticles that allow them to have higher bioavailability and concentration near the tumor mass. Nanoparticles are used in diverse applications that range from diagnosis to therapy. Gene therapy is now under development globally [3].

There are two conventional gene therapy which are transcription activator-like effector nucleases (TALEN) and zinc-finger nucleases (ZFN). These two therapies are applied to disfunction essential genes, for example, oncogenes. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated System (Cas) provides some advantages compared with ZFNs and TALENs. These involve simpler target design, higher efficiency, and multiple mutations. CRISPR/Cas system is able to induce both permanent and non-permanent alters in DNA [3].

CRISPR/Cas system is an emerging gene-editing equipment for diverse cancers' treatment. The Cas9 system employs a technique of utilizing a single programmable endonuclease to manipulate several gene functions all at once by targeting multiple genomic loci in one experiment. This technique provided broader views about pathological phenomena that are caused by several genes or mutations, helping to recognize disease-resistant genes and quickly evaluate drug targets. Therefore, CRISPR/Cas9 system may be considered promising future treatments for immunological disorders, neurodegeneration, viral infections, cancers, genetic disorders, and cardiovascular diseases. However,

there are still challenges and obstacles to a complete gene-editing technique applying Cas 9 system [3].

2. CRISPR/Cas system

2.1. Classification

The CRISPR/Cas system is first generally classified into two class-Class 1 and Class 2. The foundation of how to distinguish is that Class 1 is composed of a multimeric crRNA effector complex while Class 2 is comprised of a single crRNA effector complex. Type I, III, and IV are later subclassified under Class 1. Types II, V, and VI make up Class 2. Subtypes are later created by further dividing various types [4].

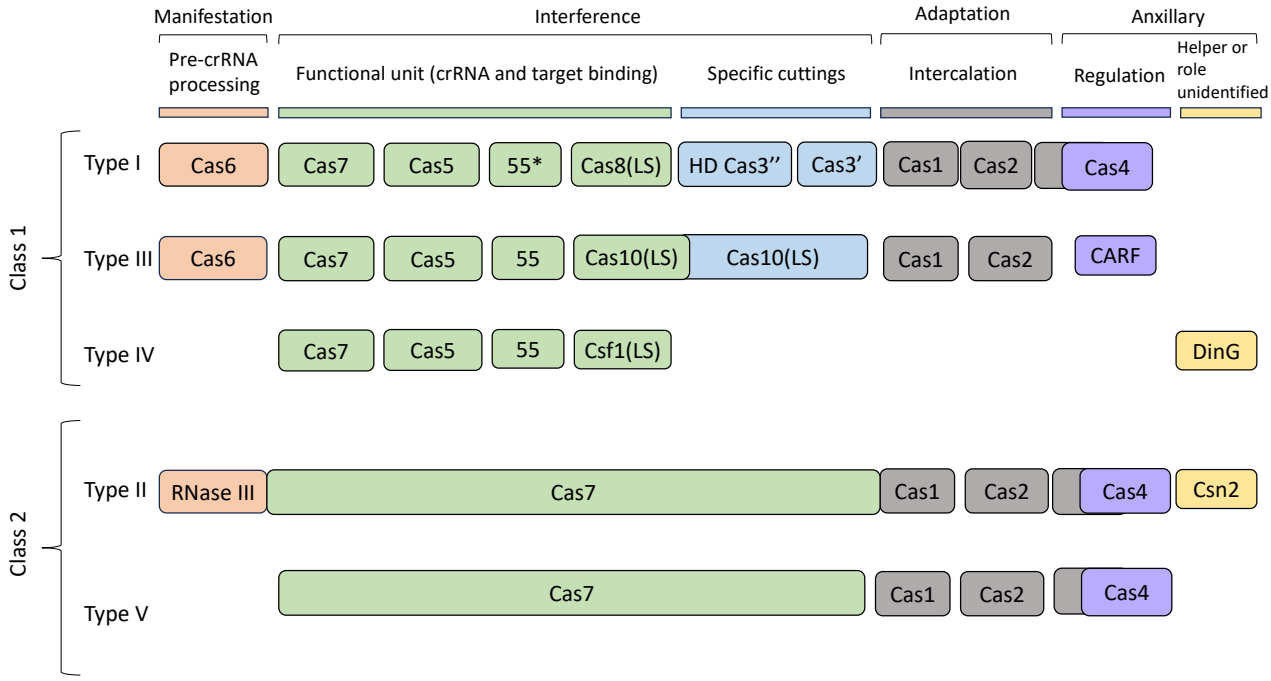


Fig. 1 The classification of CRISPR/Cas system [5].

2.2. Mechanism

Recognition, cleavage, and repair are three steps engaged in the process of the CRISPR/Cas-9 system [6]. CRISPR RNA (crRNA) is an RNA sequence that programs Cas9 to identify the target sequence. Trans-activating CRISPR RNA (tracrRNA) mediates interaction with Cas9 [7]. Designed single guide RNA(sgRNA) is applied to guide Cas-9 to recognize the target DNA sequence. Double-stranded breaks (DSBs) are formed by Cas-9 nuclease at a site where is 3 base pair ahead of PAM which is a short, conserved DNA sequence that is comprised of 2-5 base pairs. PAM, which has various sizes based on the species of bacteria, is downstream of the cleavage site. Cas-9, which is a prevalent nuclease in gene-editing tools, identifies the PAM sequences of 5'-NGG-3' which N represents any kind of nucleotide base. When Cas-9 finds the correct PAM which indicates the target site, it will cause local DNA to melt. A hybrid of RNA and DNA develops. Later, activation of the Cas-9 protein causes DNA cleavage to occur [6].

DSBs, which are exceedingly toxic, have two pathways to repair. A single mistaken repair may cause cell death, while multiple mistaken repairs can cause apoptosis [8]. There are two pathways to repair DSBs-non that are NHEJ and HDR [9].

NHEJ occurs during all stages of the cycle. The process starts with the connecting of Ku70-Ku80 (KU) and blunt DNA ends. KU later enlists other factors that are related to NHEJ, first assembling DNA-PKcs which is the subunit of DNA-PK. DNA-PKcs, which promote the DNA ends to synapse

and enable the assemble of ending enzymes, phosphorylates a wide range of substrates such as DNA ligase IV, XRCC4, and XLF. Arranged, harmonious DNA ends can be joined by the c-NHEJ factors. polymerase lambda and mu work with Artemis to get dull DNA termini ready for the following process. Finally, the XRCC4-DNA ligase XLF complex executes the joining across intervals individually. Through the operation of DNA nucleases and polymerases, DNA bases are arbitrarily removed and added. As a result, the original genetic template has microindels, which form the basis for NHEJ mending [9].

The S/G2 phase, when an accessible sister chromatid is intact, is when HDR occurs most frequently. Since information is copied from a homologous DNA duplex, HDR is a more suitable and accurate way of repairing [10].The formation of a 3' single-stranded DNA projection from 5'-to'3' excision of the end of DNA marked the change from DSB to HDR. The process is started by the MRE11-RAD50-NBS1 (MRN) complex, which also starts the excision mechanism by assembling the C-terminal-binding protein interacting protein (CtlP) [9].

3. CRISPR/Cas System in Cancer Treatment

As a developing immunotherapy for several cancers an experimental gene therapy, CAR T cell therapy readdresses T lymphocytes to diminish tumor cells [11].

3.1. CAR-T structure

As an engineered modified fusion protein that is similar to T cell receptor (TCR) in structure, one or more signaling domains inside the cells of the CAR are connected to an antigen-detecting domain outside the cells. Contrary to the first generation of CARs, which only carry the signal domain CD3 inside the cells, the second generation of CARs also carries a costimulatory molecule. One more costimulatory molecule is included in the third generation of CARs. When the tumor-associated antigen (TAA) is recognized by the CAR, the fourth-generation CAR-T cells have the potential to activate the effector transcript factor, resulting in the release of cytokines. The fifth generation of CARs uses gene editing to restrict the expression of the TCR gene, enabling the removal of TCR α and β chains. This is a crucial advancement built upon the basis of the second generation [12]. No matter what generation, CARs consist of four basic parts-intracellular signaling domain, an antigen-binding domain, a transmembrane domain, and a hinge [13].

As an essential component of CAR, the signaling domain inside the cells contains a domain for activation along with one or more co-stimulatory domains. The CAR's extracellular region contains an antigen-binding domain which is the foundation of the CAR-specific attaching to tumor-associated antigens. Antigen-binding domain mostly contains monoclonal antibodies, constitutes by a variable light and heavy chain that is connected by a flexible connector. Consequently, a single chain variable fragment is formed. The CAR's transmembrane domains and hinge play a crucial role in connecting the intracellular signaling structures and extracellular antigen-binding structural domain [13].

3.2. CAR-T therapy development

CAR T-cell therapy, the most advanced type of adoptive cell therapy, includes the therapeutic cell transfer of T-cells equipped with synthetic receptors to cancer patients. The immune system is coordinated by T cells, which also specifically target and destroy malignant or diseased cells. The treatment uses the patient's own T-cells and creates and expresses recombinant proteins on CARs using genetic engineering. Despite the existence of human leukocyte antigen or the histocompatibility complex, CARs are precisely designed to detect, target, and kill almost every cancer cell that expresses extracellular antigen [14].

3.3. Universal CAR-T (UCAR-T)

Current existing CAR-T cell therapy are all autologous. The purpose of this kind of therapy is to diminish severe rejection because of alloimmune since the major histocompatibility complex between patients and donors is mismatched. Because it utilizes heterologous CAR-T cells from healthy donors, the Universal CAR-T (UCAR-T) therapy has a variety of production costs, safety considerations, applications, and processes. The majority of preliminary and medical trials of UCAR-T therapy are used for hematological malignancies. Some targets include BCMA, CD22, CD20, and CD19. CD5, CD7, and CD70 are targets that are newly developed [15].

3.4. CRISPR/Cas9 application in CAR-T cell-based therapies

CRISPR technology offers the promise of effective immunotherapy by creating an all-encompassing "off-the-rock" biological product or changing immune cells to lessen solid or hematological malignancies [16].

Because of its relative ease and reasonable costs, the CRISPR system is more potential for manufacturing universal CAR-T cells [12].

A negative immune regulator called PD-1/PD-L1 promotes tumor cells' immunological resistance. When the PD-1 gene is suppressed in primary T cells using CRISPR/Cas9 tools that are coated with liposomes, T-cells are significantly activated and exhibit greater anticancer potential both in vitro and in vivo. CRISPR/Cas9 is an established method that inhibits PD-1 in CTLs. While CTLs with disabled PD-1 can decrease the number of regulatory T cells and employ more effector cells, CTLs with disabled PD-1 restrains tomor growth in vitro and in vivo by activating caspase and adjusting the secretion of cytokines. These cells continuously create new antibodies by antibody-mediated checkpoint blockage, which inhibits the growth of human melanoma in xenograft tumor mice [17].

3.5. Obstacle and improvement of CAT-T threrapy based on the CRISPR/Cas system

Although CAR-T therapy has ground-breaking success, it still has many challenges. (1) The manufacturing process is time-consuming and costly because CAR-T cells must be made specifically for each patient. Therefore, widespread medical uses for CAR-T cell treatment are not possible. (2) CAR-T is ineffective against cancer cells. When immune checkpoint regulators such as lymphocyte activation gene 3, CD223, or PD1 are expressed by CAR-T cells after they have been stimulated, this could prevent the anticancer effects of the cells from binding to the matching ligands produced by cancer cells. (3) Cytokine release syndrome and unwanted toxicities may occur. Concurrently activating a massive number of CAR-T cells may result in secreting high levels of IL-1, IL-6, and GM-CSF that may lead to Cytokine release syndrome [16]. (4) Patients who have solid tumors react to CAR-T therapy less frequently than patients who have hematological malignancies. The ORR for CAR T-cell treatment in patients with solid tumors was 20%, but the ORR in patients with hematological cancers was 71% .

To reduce manufacturing time, recent research has concentrated on reducing the time required for the CAR-T cell multiplication stage. According to studies, CAR-T cells harvested over a longer period of time had significantly higher anticancer activity than those obtained after three days. As CAR-T cells' capacity to produce new cells grows, they will need to be able to balance long-term function and in vivo endurability.

4. Conclusion

Cancer is a fatal illness that has been many people’s deaths cause. Current treatments and experimental treatments for cancer involve such as EV, nanomedicine, and gene therapies. Every treatment is still facing challenges and side effects while scientists are working to have more efficient therapy.

The CRISPR/Cas system, one of the gene therapies, has been a new and promising treatment that may eventually treat cancer without any adverse effects. CAR-T therapy is still developing to allow

T cells to be massively produced and transported easily. CAR-T therapy has two types of T-cell-from donors (UCAR-T) or patients themselves. This therapy still faces many challenges such as incapability of massive production, cancer cells' resistance, toxicity, and lower ability in solid tumors.

This paper introduces and evaluates the CRISPR/Cas system and its potential in CAR-T therapy. Applying CRISPR/Cas system in CAR-T cell treatment, CAR-T therapy can have higher efficiency in inhibiting tumors by blocking inhibitors such as PD-1. The altered CAR-T exhibited higher efficiency both in vitro and vivo. In the future, cancer may be cured by CRISPR system and CAR-T therapy which may decrease the death rate of cancer without side effects, lower costs of cancer treatment and cure millions of people.

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