

CRISPR-Cas9 and Immunotherapy for Cancer

Yifei Zhong *

School of Long Chang No.1 Middle School, Neijiang, China

* Corresponding Author Email: YifeiZhong4398@outlook.com

Abstract. Genome editing is part of the key research topics today. Researchers found, that the system of CRISPR-Cas9 can use Cas9 protein to manipulate the actual gene parts, playing a genuine editing role. Nowadays, the system of CRISPR-Cas9 is using widely in many area, like the cultivation of genetically modified crops, industrial production and the treatment for illness and so on. Except for those, the CRISPR-Cas9 system can be also with other method like immunotherapy to achieve the treatment in different types of cancers. In the development of specific cancer, they achieve continuous proliferation by capturing the host immune checkpoint. With the aberrant expression of PD1 in cancer cells, they achieve immune evasion through interaction between PD1 and PD-L1, which are currently the target of cancer treatment. This paper utilize the treatment of non-small-cell as an example to expound the knockout of PD-1 by CRISPR-Cas9 system, artificially constructed T-cell can keep activating T-cell proliferation.

Keywords: Cancer; CRISPR-Cas9; Immunotherapy.

1. Introduction

Cancer goes hand in hand with the aggregation of malignant cells in local or global location. After proto oncogene that control cell proliferation is aggrieved and changed into dysfunctional structural conformation, which are oncogene, it's deficient in controlling cell proliferation, leading to uncontrolled cell growth and division. Meanwhile, if the DNA repair pathway did not work and can not repair DNA damage, cells are potential to elaborate the ability of achieving immortality by against apoptosis. Besides, the characteristics of cancer cells also contain unidirectional growth, quantities of blood vessel development and the capability of evading cell growth control. Except for these, compared to normal cells, the key feature of cancer cell is its metastasis, transferring of tumor cells from the primary location to further aim location or even throughout the whole body. The reason for cancerous morbidity is very complicated. Ingestion of chemical such as alcohol ,benzene and heavy metal, unhealthy eating habits, fat and lack of exercise, high-frequency radiation, pathogen infection, inheritance and cells' internal cause are the common risk factors resulting in canceration. Cancer can take place in almost everywhere through the whole body, like gastric cancer, hepatoma,intestinal cancer, osteocarcinoma and leukemia. Generally, each category of cancer has big harm for individual, leading to the dysfunction of metabolism and ruin of organ function, eventually resulting in high mortality. During the period of disease occurrence, patients always suffer from serious physical and economic burden. Currently, the curative treatment to cancer include traditional surgical treatment, interventional therapy, radiation therapy, chemistry therapy and new targeted therapy, immunotherapy and so on. The application of surgical treatment depends greatly on the patients' body circumstance, only effective for early stage malignancy but not metastasis, in addition, the lesion location and area also control usage. Interventional therapy can control tumor proliferation, development and spread. It can also treat complications, but it is just be aimed at regional treatment about tumor. Radiation therapy is a treat way utilizing superior energy to die out cancer cells directly. Through a lump of high energy particles, it bombards cancer cells directly, destroying DNA or killing cancer cell by generating free radicals through ionization. Chemistry therapy is widely used in clinical practice but have serious by-effect like myelosuppression, puke, gastrointestinal reaction, hair fall off, dry skin and pain and so on, limiting its application in all kinds of malignancy. Immunotherapy is a hot topic in recent years. It is a very powerful treatment and won the Nobel Prize in Physiology or Medicine in 2018. Basically, the immunotherapy is stimulating or enhancing the host immune response to overcome pathogens. One of the immunotherapy is extracting patients' immune cells out

of the body and manipulating in vitro, then, filling them back to patient's body to rescue patients' immune ability and kill tumor cells. Currently, the cell-based immunotherapy is mainly classified into two categories including CAR-T cell therapy and NK cell therapy. Traditionally, CAR-T is just an artificial T cell incorporating antigen recognition segment. Nowadays, with the discovery of immune evasion through immune checkpoint, a recent CAR-T therapy developed. Firstly, the PD-1 gene within in T-cell is, then taking them back to patient to stimulate immune ability and kill tumor cells. In clinic, the manipulation of T-cell is done by genetic engineer, mainly CRISPR-Cas9, which is the focus of this review.

2. Main body

2.1. The mechanism of PD-1 and PD-L1

Naturally, PD-1 is broadly expressed in functional T cells and locate in the cell membrane. It can speed up the maturity of T lymphocytes, regulate the activity of T-cell by negative feedback, control the immune response, and promote self tolerance. Once binding of PD1 to PD-L1(Programmed cell Death-Ligand 1), the proliferation of PD-1 positive cells is inhibited, what's more, the secretion of cytokine is reduced and the apoptosis of T cells occur. In native state, the PD1/PD-L1 is in charge of controlling the immune response under physiological level, while, some cancer cells achieve immune escape by this mechanism [2]. As the PD1 is expressed on the surface of tumor cells, after binding to PD-L1, the production of active T cells is heavily ceased and the death of mature T cells is initiated, all of those leading to the decrease of IL-10(Interleukin 10) generation, finally immune suppression [6].

2.2. CRISPR-Cas9

2.2.1 CRISPR-Cas9 system

CRISPR called Clustered regularly interspersed short palindromes repeats in total. CRISPR system existence in germ in natural. By the CRISPR system, host can remember pathogens who attacked them through the pathogens derived nucleic acids sequence like acquired immunity in human. CRISPR-Cas9 technology is co-created by Emmanuelle Charpentier from French and Jennifer Douna from America and win Nobel Prize in Chemistry in 2020. Emmanuelle Charpentier and Jennifer Douna found CRISPR-Cas9 system by accident when they are researching the immune system of streptococcus. They concluded that it is a separate numerator tool that can precisely cut genetic material, making changing of life code come true. As a genome editing tool, the CRISPR-Cas9 system is primarily composed the Cas-9 protein and the guide RNA (gRNA).

2.2.2 The mechanism of CRISPR-Cas9 technology

GRNA, a sequence composed of about 20 nucleic acids, which is complement to the target site. It can bind to the cas9 protein and guide it to the exact position by base pairing, resulting in genetic modification, achieving knock-in and knock-out. Experimentally, a plasmid containing the cas-9 and gRNA encoding sequence is transformed into cell, then, they are translated within cell. With the guidance of gRNA, the endonuclease Cas-9 protein is placed near the target location. The interaction between Cas9 and dsDNA is mediated by its Oligonucleotide binding domain (OBD) or PAM Interaction Domain (PID). After the interaction, cas9 protein scans in the sequence and finds the PAM sequence, then, cutting on the third basic group where on the upstream of PAM until the last paired site. The cut of Cas-9 lead to the generation of DNA double strand break (DSB) in target gene, which is identified by the DNA repair system. DNA repair mechanism is divide into Non-homologous end joining (NHEJ) and Homology-directed repair (HDR).NHEJ is rapid and high efficiency, but will randomly causing deletion mutation, making target sequence no longer be transcribed and translated normally. HDR is a error-free repair mechanism, but need the homolog template, additionally, it's less efficiency compared to NHEJ.

2.3. The application of CRISPR-Cas9 and immunotherapy of cancer--Take non-small-cell lung cancer as an example

2.3.1 Principle

As mentioned before, the CRISPR-Cas9 is an efficient and precise tool to modify target genes without sequence preference. In the manipulation of PD-1 gene within T-cell, the PD-1 is knockout, eliminating the interaction between PD-1 and PD-L1, which can rescue immune suppression caused by PD-1 progressive cancer cells [7].

2.3.2 Process

Non-small-cell lung cancer is a prevalent cancer type and accounts for 85% lung cancer patients. Non-small-cell cancer is further categorized into adenocarcinoma, squamous cell carcinoma and large cell lung cancer. They occurred separately in lung surface bronchus and bronchiole or alveolus pulmonic. The treatments include surgical treatment, radiation therapy, chemotherapy, targeted therapy, immunotherapy and clinical tests. Recently, with the advance of genetic engineer technology, it has been also applied in therapy, especially immunotherapy. For instance, professor You Lu from Hua Xi hospital of Sichuan University utilizes CRISPR-Cas9 technology to treat non-small-cell lung cancer. However, through NGS and WGS, they found that larger scale of mutations, including frame shift, non code shifting(lost/insert mutation) and stop-obtain mutation, occurred after genetic modification. Totally, the mutation within intron and protein coding region together make up approximately 83.2% match [7]. So, we should check up CRISPR-Cas9 system in experimental operation or clinical trials on time to prevent miss the target in shooting practice.

2.4. Miss the target in shooting practice

2.4.1 Mechanism

Cas-9's Targeted fidelity is determined by the 20nt sgRNA and PAM which near Genome targeting sequence. When the off target effects interference with chromosome stability, it may cause functional genomics losing their activities and various physiological or signaling abnormalities. While, it has low possibility to prevent off target effects' happen by designing best sgRNA and achieving high targeting precision considering its short range [8].

2.4.2 Off-target detection method

Although it's difficult to prevent the occurrence of off target directly, it's helpful to detect the off-target effect timely to estimate the safety of it. The sequence's non specificity cutting activities depend on the target sequences' homology, the interact with nucleic acid enzyme's numerator with it and the accessibility itself. To analyze the off target effect, the first step is determining the possibility of off target in genome by external tools or whole genome identification method, then, counting the insertion/deletion in prediction site by quantitative detection technology or next generation sequencing. The common test method include the nucleic acid enzyme conjugate detection, nuclease induced DSB detection and detection of repair products after induction of DSB by nucleases. Although the nucleic acid enzyme testing is most simply to testing, but nuclease requisite is necessary and insufficient, so, it might result in high quantities of false positive, which limits its broad usage. SITE-seq and CRICLE-seq are two DSB detection strategies. The defect of them is that they ignore the chromatin's status in activity cells, which leads to plenty of false positive event as well. The GUIDE seal technology is another way to identify potential off target site in many laboratory. GUIDE seal determines the off target activity by introducing short double stranded oligonucleotides(dsODN) when nuclease cleavage occurs. The main defect of GUIDE seal is that it needs dsODN to be delivered into cell but not all kinds especially primary cell(as shown in table1.comparision of predictive performance of off-target site search tools in experiment). Except the method of testing nuclease off-target's activities, the second generation sequencing technology(Amp-NGS) can detect the mutation caused by off-target directly. Amp-NGS has high sensitivity and can be applied to any sample, usually taken as the effective strategy on testing nuclease off-target cutting. However, Anp-NGS will be

affected by the PCR and NGS' mistake in sensitivity and disturb the testing on actual cutting event. As the situation with NGS' reading mistake is base substitution, and NHEJ repair nuclease induced DSB technology will cause short InDel, so, Amp-NGS quantization by the frequency about insertion/deletion not base mutation frequency. By the mistake from PCR and sequencing error, there are a number insertion and deletion rate of non target punctuation marks will exceed 0.1% which official sensitivity about Amp-NGS. So we should set up destructive control to define its really background signal when using Amp-NGS to analyse [9].

Table 1. Comparison of predictive performance of off-target site search tools in experiment

Name	Method	What is detected	Setting	Cut site enrichment	Cellular false positives
In vitro					
Digenome-seq 47	NGS fragment end statistics	DSB	DNA	170	65 %
Circle-seq 49	DSB end enrichment	DSB	DNA	821	88 %
Site-seq 48	DSB end enrichment	DSB	DNA	178	95 %
Dig-seq 51	NGS fragment end statistics	DSB	DNA	289	64 %
In vivo					
HTGTS26	Rearrangement detection	Repair product	Cells	4,700	n.d.
Guide-seq27	Oligonucleotide integration	Repair product	Cells	29	20 %
Discover-seq55	DSB end enrichment	DSB	Cells/tissue	233	~0 %
BLISS54	DSB end tagging	DSB	Cells/tissue	160	20 %

3. Discussion

CRISPR-Cas9 is a type of genome editing tool with moderate efficient and its development prospects are very broad. As shown in the application of CRISPR-Cas9 with CAR-T therapy in non-small-cell lung cancer treatment, its ideal in total, but still has the situation on off-target. So, first, we should devise the sgRNA more precise. Second, we should intensify supervision efforts and check the Cas-9 protein's combine situation in time. CRISPR-Cas9 may cause an overactivated immune response and transgenation by off target, accompanied side effects include cytokine release syndrome (CRS), neurotoxin, cancer recurrence and resistance. For these values, we need to reduce off-target incidence, closely monitor changes in the condition, promptly detect and handle allergic reactions. Only in this way, can the CRISPR-Cas9's technology will be improved with less off target, higher safety, and broader application in clinical treatment.

References

- [1] JHUNJHUNWALA S, HAMMER C, DELAMARRE L. Antigen presentation in cancer: Insights into tumour immunogenicity and immune evasion [J]. Nature Reviews Cancer, 2021,21(5):298-312.
- [2] LI X S, LI J W, LI H, et al. Prognostic value of programmed cell death ligand 1 (PD-L1) for hepatocellular carcinoma: a meta-analysis [J]. Bioscience Reports, 2020,40(4): BSR20200459.
- [3] BLANK C U, HAINING W N, HELD W, et al. Defining 'T-cell exhaustion' [J]. Nature Reviews Immunology, 2019,19(11):665-674.
- [4] PHILIP M, FAIRCHILD L, SUN L, et al. Chromatin states define tumour-specific T-cell dysfunction and reprogramming [J]. Nature, 2017,545(7655):452-456.

- [5] JIMBU L, MESAROS O, NEAGA A, et al. The potential advantage of targeting both PD-L1 / PD-L2 / PD -1 and IL-10 – IL-10R pathways in acute myeloid leukemia [J]. *Pharmaceuticals*, 2021,14(11):1105.
- [6] Jennifer A. Doudna et al. Diverse virus-encoded CRISPR-Cas's systems include streamlined genome editors *Cell*,2022, 185(24): 4574-4586.
- [7] You Lu et al.Safety and feasibility of CRISPR-edited Tcells in patients with refractory non-small-cell lung cancer *Nature medicine*,2020,0845(5):732-740
- [8] Hakim Manghwar et al.CRISPR/Cas Systems in Genome Editing:Methodologies and Tools for sgRNA Design,Off-Target Evaluation,and Strategies to Mitigate Off-Target Effects *Advanced Science*,2020,7(6): 1902312
- [9] Bao XR,Pan Y et al.Tools for experimental and computational analyses of off target editing by programmable nucleases *Nature protocols*,2021,16(1):10-26