Dean&Francis
ISSN 2959-409X

CRISPR/Cas9: A Brief Introduction and Applications in Cancer

Yuan Yao[1, *]

[1]Department biology faculty, Shenzhen MSU-BIT University, Shenzhen, China

*Corresponding author: 1120210248@smbu.edu.cn

Abstract:

CRISPR/Cas9 is a relatively new technology for gene knockout developed from prokaryotic immune mechanisms against exogenous DNA. The technology has been extensively used in cancer research and therapy development. CRISPR/Cas9 as a relatively in-mature gene editing tool with certain limitations, has shown great potential in applications in a variety of fields. In this review, a basic and brief introduction of the technology and its application in cancer research and therapy development will be given. This includes the principles and development of the technology, genetic screens using CRISPR/Cas9 finding new therapy targets, its use in gene therapies, its role in creating modified cells immunotherapies, some works that have been done in these fields and the future possibilities of the technology. This review aims at providing a basic and rudimentary understanding of CRISPR/Cas9 as well as its applications in cancer. And perhaps give some insight for future studies regarding the technology and its applications in cancer.

Keywords: CRISPR/Cas9; gene editing; development; cancer.

1. Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) is a kind of gene that is commonly found in bacteria and archaea. It's an important part of archaea and bacteria immune system against exogenous DNA fragments. With Cas (CRISPR associated) proteins, CRISPR-Cas system is capable of identifying a specific gene and deactivate it. This immune mechanism has been adapted to be used as a genetic modifying tool known as CRISPR/Cas9, Cas-9 is one of the Cas proteins that is used in this technology. CRISPR/Cas9 provides a simple and convenient way to deactivate specific genes and has been used extensively in recent years [1].

Cancer has always been the grand trophy of medicine. As the average lifespan of humans continues to rise, cancer has become the major cause of human death, and the defeat of cancer has become the problem of our time. In recent years, new treatments such as target therapy and gene therapy have been developed and put into use and many newly developed technologies have been put into solving this problem of our time, so as CRISPR/Cas9 [2].

In CRISPR/Cas9, a plasmid containing the gene directing the synthesis of Cas-9 complex and a fragment of the target gene (target seeking sequences)

ISSN 2959-409X

is introduced into the target cell, and then Cas-9 complex which consists of Cas-9 protein and sgRNA is synthesized in the target cell. The target seeking sequence on sgRNA will then combine to the target gene and the Cas-9 complex will deactivate the gene by removing part of its sequence from it [3,4].

One of the two major CRISPR/Cas9 applications in cancer is its use in identifying potential targets for new target treatments. By deactivating genes in tumor cells in vitro and evaluate the effects of such deactivations on the growth of tumor cells, genes of great importance to tumors can be identified and the protein that it encodes can be recognized as a potential target for therapy developmont. A number of studies have been done to identify therapy targets using this method. Besides the use in finding new targets for targeted treatments, CRISPR/Cas9 can also be used in treating the disease. Attempts of introducing genetic modification T-cells into mice bodies in order to attack tumor cells have been made to examine the possibility of such treatments. CRISPR/Cas9 is used to develop these cells. CRISPR/Cas9 can also be used directly to treat cancers by manipulating genes of certain groups of cells in the body including tumor cells. This review aims to provide a basic introduction to the principles of CRISPR/Cas9 and briefly introduce the two major types of CRISPR/Cas9 applications in cancer and some of the work that has been done in this field.

2. The Principles and Development of CRISPR/Cas9

The CRISPR was first discovered over 30 years ago in E.coli K12. CRISPR is a construction in which a number of repeated DNA sequences are separated by non-repetitive sequences of similar lengths. After its discovery, CRISPR was found to exist in many other organisms. It was found that around 36% of bacteria and around 75% of archaea carry such DNA constructions [5]. However, at that time the function of CRISPR was not understood. Later in 2005, a breakthrough was made in this field. Parts of CRISPR were found to have foreign origins including bacteriophages and plasmids, the relation between the existence of CRISPR and the organism's ability to resist certain viruses and plasmids was also revealed, those containing viruses or plasmids originated sequences in CRISPR exhibit resistance to those viruses or plasmids [6-8]. Till this point, it has been confirmed that CRISPR is related to an immune system against exogenous DNA, extensive researches were made to discover its mechanism. cas9 was found to be the executing unit of the system, this protein exhibits nuclease activity under the guidance of short CRISPR RNAs (crRNA) [9]. Then in 2011, trans-encoded small RNA (tracrRNA) was found to be responsible for the activation of cas9 DNA cutting [10]. It was made clear that CRISPR cas systems relies on tracrRNA binging to their matching sequences to identify targets and trigger responses.

After the discovery of CRISPR cas system, it is quickly realized that the system has a potential of being used as a genetic editing tool. By editing CRISPR sequences, the system can be directed to deactivate target genes.

In 2013, multiple groups declared that they had successfully achieved gene editing in Eukaryotic cell using this system [11-15]. This is the birth of a revolutionary technology and this technology has greatly influenced the field in the last decade.

The greatest focus and the key step of this method is its delivery. CRISPR cas system can be delivered into target cells in multiple forms including DNA, RNA, and RNP. And the delivery approach is very diverse, including physical methods and delivery using viral vectors or non viral methods like plasmid. These methods have their advantages and disadvantages and the specific approach that should be used depends on actual conditions.

Physical approaches like microinjection and electroporation are simple and straightforward with high transfection yields. Content can be delivered in multiple forms. However, such operations can be very harmful to the subject cells, some specific cells in particular and for that reason this delivery method is limited in using scenarios and is not an option in some cases [16].

A more popular way of delivery is using plasmids. Sequences encoding the synthesis of cas9 complex are introduced into target cells on plasmids. The problem with this method is the possibility of plasmid DNA integrating into the genome of host cells, which could cause unexpected DNA cuts, also, in some cases plasmids could trigger immune response or other unwanted reactions in host cells [17].

Another approach is the use of viral vectors like AAV. These vectors can effectively deliver RNA/DNA contents into target cells but they are limited in delivery capacity. AAV vector can carry contents up to 4.7kb and gene encoding SpCas9 alone is about 4.2kb, this undoubtedly limits further modification. Besides this, viral vectors also have the problem of integrating and they will most certainly trigger immune responses [18].

Besides delivering RNA or DNA, another approach is to deliver RNP, which is directly introduce working Cas9 complex into the target cell. Although this method has a short turn effect, but the accuracy and efficiency are substantially higher.

The existing methods all come with some disadvantages and the delivery of CRISPR cas system is still to be fur-

ther explored.

3. Gene Screening and Target Identification Using CRISPR/Cas9

As a powerful tool for knocking out genes, CRISPR cas9 system can be used to determine the function of a gene by deactivating it and observing the consequences of such editing. CRISPR/Cas9 has already been extensively used in gene screening and has proven to be a very powerful tool in gene screening [19-22].

Cancer is a disease caused by genetic mutations of certain cells, which makes the development of its treatment greatly related to our understanding of the genes related. Studies have been done to screen tumor related genes using CRISPR/Cas9, For example a series of genes including PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha), KRAS (KRAS Proto-Oncogene), SMAD4 (SMAD Family Member 4), TP53 (Tumor protein P53), and APC (Adenomatous polyposis coli) in cells found on organoids of human intestinal epithelium were processed using CRISPR/Cas9, the edited cells were introduced into mice bodies and formed tumors in mice, this research helped to understand the roles of these genes in human colorectal carcinogenesis [23].

Target therapy is one of the most popular new approaches to treating cancer and has been considered to be one of the most promising methods in treating cancer. One of the primary benefits of CRISPR/Cas9 gene screens is that certain genes can be located, which could cause tumor cells to lose activity or deactivate when missing. These tumor affecting genes could potentially represent new targets that can be developed into new target therapies [23].

Besides screening tumor cells, T-cell screens are also potential target sources, understanding genes that affect the activity of T-cells could potentially reveal new approaches to treatment, For example, research using CRISPR/Cas9 to screen T-cell genes has revealed a new factor that inhibits the activation of T-cells, which could potentially be a new target in treatment development [24].

These new research methods based on this technology have proven to be very effective and powerful tools in designing new target therapies in the future. As the methods continue to develop and researches continue to be done, CRISPR/Cas9 will undoubtedly greatly contribute to future cancer treatment development.

4. Clinical Applications of CRISPR/Cas9 Use in Gene Therapy

Current clinical applications of such technology can be roughly divided into two types, direct and indirect. Indirect applications include use of cells taken from subject bodies that are genetically modified in vitro and then reintroduced into subject bodies to function. In the direct application however, cells are genetically modified in vivo. Editing agents are directly delivered into subject's body and transform the target cells. In this part, a basic introduction of the use of CRISPR/Cas9 in both types will be given.

CRISPR has been used in several cases as the tool to modify genes that causes diseases. In one of these attempts, CRISPR vector designed to correct a mutation which is closely related to cancer was sent into mice through vein and carried to liver of the mice by blood, as a result 20% of hepatocytes were successfully transformed [25]. For cancers that are related to mutations, such methods can be used as a treatment. Burkitt's lymphoma is a cancer that is caused by a specific mutation. An attempt of treating it using CRISPR/Cas9 has been made and in result reduced tumor proliferation [26]. similar research using other gene editing tools has also been made. For example, TALENs were used on a one year old patient to treat leukemia and it was a successful attempt, such successful applications of gene therapy indicate the potential further uses of CRISPR/Cas9 in clinics [27]. Another way of using CRISPR/Cas9 to treat cancers is targeting cancer cells. Cancer cells are mutated somatic cells. Through manipulation of the genome of cancer cells, an effective treatment could be achieved by delivering CRISPR/Cas9 vectors to the tumor [28].

The other form of gene therapy is the use of in vitro transformed and grown cells. Immune cells, T-cells in particular are mostly used in such processes. Methods of using modified immune cells to fight tumors are currently being studied. In these studies, two major types of T-cells are being used, TCR-T cells (T cell receptor) and CAR-T cells (chimeric antigen receptors).

CAR is a recombinant antigen receptor. These receptors enable T-cells to identify and attack designated targets and start intensive anti tumor responses [29]. Typically, T-cells are extracted from subjects, transformed into CAR-T cells and expanded in vitro, then reintroduced into subject's body [30]. This process is for avoiding the immune response to exogenous T-cells. However, having to use T cells from each specific patient is a significant disadvantage of this method, not only it will take a substantial amount of time to prepare T cells, it also greatly depends on the conditions of patient's T-cells.

If allogeneic T cells can be used, it will not only solve these problems but also considerably reduce the cost [30]. In 2012 an attempt of genetically modifying CAR-T cells to avoid immune response from the host using ZFN to remove a specific gene was successfully made [31]. This

ISSN 2959-409X

indicates gene modifying tools like CRISPR/Cas9 are powerful tools in developing CAR-T therapy. Using the CRISPR system to direct CAR to the TRAC locus can effectively delay differentiation and exhaustion of effector T cells [32]. Such enhancements represents the potential uses of gene editing tools like CRISPR/Cas9 in CAR-T development.

TCR-T cell is another type of cell that is used in fighting tumors. It is considered that TCR-T cells are superior in dealing with solid tumors compared to CAR-T due to its features including a larger variety of antigen identification. The problem of TCR-T lies mainly on interactions between exogenous TCR and pre existing TCR, this significantly reduces the expression and the effectiveness of exogenous TCR [33]. To deal with such problem, CRISPR/Cas9 is used in a research to knockout endogenous TCR of T cells, in result, the enhanced TCR-T cells exhibited significantly superior performance [34].

Other genes can also be knocked out with the technology to enhance the performance of TCR-T. Interfering immune checkpoint genes can reduce the genes' inhibitory effects and substantially increases its anti-tumor abilities [35]. And removing cytotoxic T lymphocyte-associated antigen 4 molecules can also improve the anti-tumor performance of cytotoxic T lymphocytes in bladder cancer [36].

5. Future Potentials

CRISPR/Cas9 has been extensively used in the development of a variety of treatments of cancer. As a gene editing tool, CRISPR/Cas9 is a very powerful tool in manipulating genes and exploring the functions of genes. As future understanding of genes related to cancer grows deeper and deeper with the help of CRISPR/Cas9 technology, it could become the foundation of the development new therapies. And as the technology continues to perfect editing regarding cancer related genes can be made in advance to prevent cancer. Such potential precautions could to reduce the chance of cancer, but the ethical concerns cannot be ignored, any development of gene editing must not be proceeded without full discussion of ethical concerns. CRISPR/Cas9 uses on tumor cells are on the other hand have less concerns of such risks, as the delivery of CRISPR/Cas9 becomes more efficient and safer in the future, gene therapies using CRISPR/Cas9 have the potential of becoming a rising new option in treating cancers.

6. Conclusion

CRISPR/Cas9 is a gene editing system developed from an adaptive immune system of prokaryote against exogenous DNA fragments. As a new technology, it remains to be perfected. The focus of its development is primarily on the delivery strategy. However, being a very powerful tool in manipulating genes with many advantages has made it very popular and its applications can be found in a variety of scenarios. The application of CRISPR/Cas9 in cancer mainly focuses on genetic researches related to cancer which could potentially lead to the discovery of new therapy targets and gene therapies and applications in gene therapies including direct use and indirect use in developing CAR-T and TCR-T therapies.

CRISPR/Cas9 has demonstrated its potential of applications in a variety of fields. For cancer related studies, it could help in both disease research and therapy development. This technology could play an important role in the growth of our understanding of the disease and the development of new treatments as a basic tool. However, the technology also has imitations, it can only remove DNA fragments from the target sequence due to its nature of nuclease. As mentioned, its delivery methods are still being perfected.

This review gives only a very brief and basic introduction of the technology and its applications in cancer. Descriptions have been made as simple and straightforward as possible and a lot of details are not included.

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