Leukemia Treatment Based on CRISPR Genome-Editing Technology

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Abstract. By reviewing the literature and summarizing and refining it, this research investigated the various ways in which CRISPR gene editing technology can treat the leukemia pathology, and this research will look forward to the future application of CRISPR-based gene editing technology. At present, there are two main ways to apply CRISPR technology to influence leukemia treatment. The first is to directly knock out a known disease-causing gene to inhibit the proliferation of harmful cells, thus delaying the disease and realizing the purpose of leukemia treatment. The other is to indirectly inhibit leukemia by inserting a certain gene through the CRISPR gene editing technology to achieve the inhibition of the expression of disease-causing genes. However, the current direction of research by using CRISPR technology to explore the pathogenesis of leukemia and disease-causing genes is relatively small. In the future, more research and experiments can be conducted to explore the pathogenesis and causative genes of leukemia by applying CRISPR technology.

Keywords: Leukemia, CRISPR, genome-editing technology, treatment.

1. Introduction

Leukemia is a class of malignant clonal diseases of hematopoietic stem cells, and the occurrence and the development of which are tightly related to chromosomal and genetic abnormalities [1, 2]. Leukemias are mainly classified into acute and chronic leukemias, which can be further divided into myeloid and lymphoblastic leukemias. Most leukemias are caused by one or more genes or chromosomal translocations, which lead to malignant cell proliferation and inhibit normal hematopoiesis. For example, the vast majority of patients with chronic myelogenous leukemia (CML) will have a defect in the chromosomes of the bone marrow cells, and this chromosomal defect is mainly due to the translocation of the abl gene on the body's chromosome 9 to the bcr on chromosome 22, resulting in the formation of the bcr/abl fusion gene.

At present, there are different treatments for leukemia, such as chemotherapy and stem cell transplantation. However, there is no universally appropriate treatment for leukemia because of the complexity of leukemia typing and prognostic stratification, and it is necessary to combine the detailed typing and prognostic stratification to formulate a treatment plan. The CRISPR-Cas9 system is an adaptive immune system present in a wide range of microorganisms, and it can be used as a fast, cost-effective, high-efficiency and scalable tool to manipulate genomic sequences [3]. Currently, the CRISPR/Cas system, a revolutionary gene editing tool, was broadly used in various researches.

CRISPR-Cas systems are used for the editing of eukaryotic DNA, for knockout, replacement, epigenetic editing of DNA sequences and for activation and inhibition of gene expression [4]. This provides a new potential therapeutic target for precision treatment of leukemia. However, there are limitations to this gene editing technology, and understanding how CRISPR/Cas gene editing works can help us achieve the goal of precisely modifying genes and achieving successful treatment of leukemia.

In recent years, more and more research in the study of leukemia pathogenesis and treatment began to apply CRISPR technology. The earliest is through the use of CRISPR method to target the interference gene sequence, so as to achieve the purpose of breaking the blockage of acute promyelocytic differentiation to achieve the purpose of treatment of the disease. But in the end, although successful interference with the gene, but it is not certain that the CRISRP/Cas9 system successfully interfered with the fusion gene cannot be formed so as to cause the differentiation

blocking effect disappeared conclusion [5]. With the increasing maturity of CRISPR technology, more and more studies on leukemia have begun to apply this technology. A CRISPR/Cas9-sg RNA genome-wide library, screening on human monocytic leukemia cell lines, was developed. The use of CRISPR/Cas9 gene editing technology to edit a cell line with the DNMT3A mutation, its DNA extraction products can be used as internal quality control and external quality evaluation materials for acute myeloid leukemia (AML) single-gene testing, and the mutation can be stably inherited [6]. The use of CRISPR-Cas9 gene editing technology HOXA5 gene can significantly affect the proliferation ability of AML cells [7].

This research will shortly discuss the development of CRISPR gene editing technology in recent years and its application progress in leukemia therapy, and also discuss the challenges faced by gene editing technology in the clinical application of leukemia therapy.

2. CRISPR technology in leukemia treatment

The CRISPR/Cas9 technology can be used to target interference with the sequence of PML gene, affect the formation of PML-RARa fusion gene, break it to block the differentiation of acute promyelocytic leukemia to achieve the purpose of disease treatment. The CRISPR/Cas9 plasmid was successfully constructed and transfected into 293T cells to interfere with the PML gene. However, it cannot be concluded that the CRISRP/Cas9 system successfully interfered with PML, preventing the PML-RARa fusion gene formation and resulting in the loss of the differentiation blocking effect [5].

CRISPR/Cas9 technology can be also used to knock down the LSD1 gene of K562 cell line. Single cells were obtained by flow cytometric cell sorting technique, and one LSD1+/- and LSD1-/- cell line was obtained by expansion and culture, Western blot and sequencing identification respectively. They used MTS method to detect the effect of LSD1 knockdown on K562. The effect of LSD1 knockdown on K562 cell proliferation was detected by MTS method. The effect of LSD1 on the expression of K562 cell surface markers was detected by flow cytometric analysis. LSD1+/- and LSD1-/- cell lines of K562 were successfully constructed. Knockdown of LSD1 significantly inhibited the proliferation of K562 and the expression of CD235a, a sialic acid glycoprotein localized on the surface of human erythrocytes, which is a specific M and N blood group carrier [7]. Most of the erythroleukemia in neoplastic nucleated erythrocytes express this protein, while acute myeloid leukemia and acute lymphoma leukemia hardly express blood group glycoprotein A. Therefore, it can be used in the clinic to aid in the diagnosis of erythroleukemia and to identify red lineage leukemias. And LSD1 gene was found to have a key regulatory role in the proliferation of K562 cells and the expression of CD235a [8].

CRISPR/Cas9 system be able to terminate the expression of avian endogenous leukemia virus ALVE1 and its function. The researchers knocked the transcription termination sequence (SV40ployA) into the promoter region of the endogenous retrovirus ALVE1 by CRISPR/Cas9, terminated its transcription to realize the de-activation of ALVE1, successfully constructed a new method to study the de-activation of poultry endogenous retrovirus, providing a new idea to study the function of endogenous retrovirus indirectly. ALVE1 deactivation, successfully constructed a new method to study the deactivation of endogenous retroviruses in poultry, which provided a new idea for the study of the function of endogenous retroviruses, and indirectly realized the inhibitory effect on leukemia [9].

The researchers also proved that the other genome editing nuclease, such as ZFN nuclease, enabled the removal of the BCR/ABL1 oncogene [10], as shown in Fig. 1. The use of a pair of ZFNs targeting BCR exon 1 created a premature stop codon that triggered truncated oncoproteins. The ZFN-edited cells had a higher rate of apoptosis and lower proliferation. The group then published a study in which they addressed the technical limitations associated with the use of ZFN [11]. The experiment inhibited the transcription of oncogenes by indirect means, employing a novel CRISPR RNA-guided FokI nuclease (RFN)-based strategy to target ABL1 exon 2. This could avoid the limitations of last systems. RFN editing has successfully decreased the expression of BCR/ABL1 and its downstream targets in

both imatinib-sensitive and imatinib-resistant forms of K562. The cells lost their original malignant potential after being edited, suggesting a reduced capacity for proliferation and colony formation in vitro. After transplantation of the edited cells into the tail vein of immunodeficient NOD/SCID animals by intravenous injection, they exhibited impaired leukemogenic capacity [12].

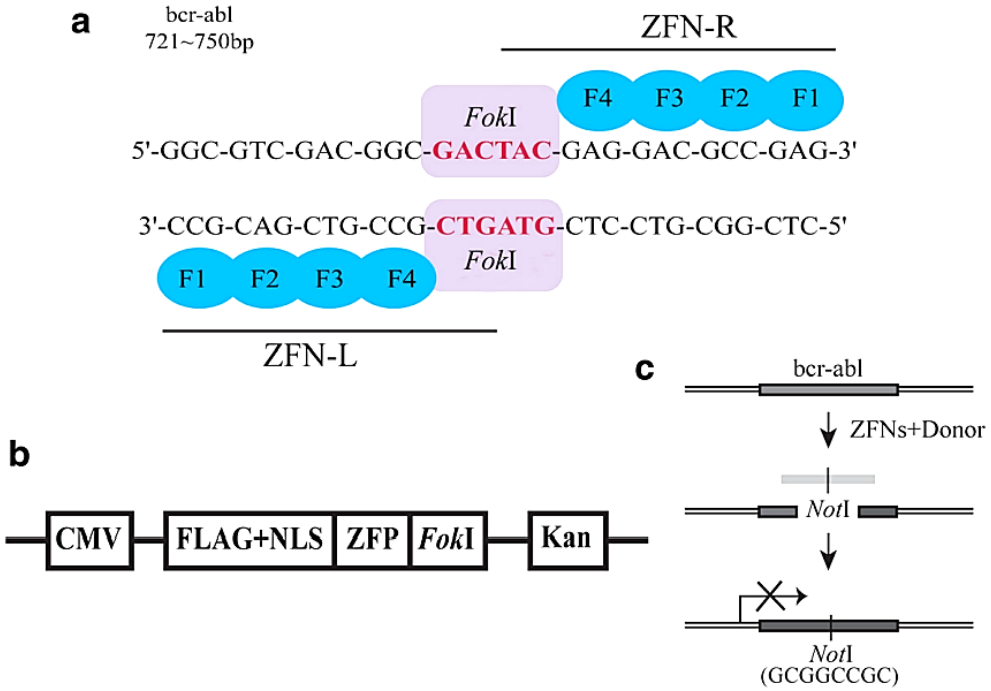


Figure 1. Application of ZFNs to target BCR-ABL gene [10]. (a) Targeted sequence of ZFNs on BCR-ABL gene. (b) The structure of pAd-Track-ZFN vector. (c) Donor construct and HDR detection

CRISPR/Cas9 was applied to knockdown miR-21 gene in human CML cell line K562 and its Imatinib (IM)-resistant strain KG, and miR-21 knockdown single-cell clones were constructed to investigate the effectiveness of CRISPR/Cas9 in knocking down the microRNA gene in hematological tumor cell lines.

Current state-of-the-art research in China is using CRISPR-Cas9 gene editing technology HOXA5 gene can significantly affect the proliferation ability of AML cells [7]. Firstly, the sgRNA sequence of the gene of HOXA5 was designed in the website, then the vector was constructed, the vector plasmid was recovered, the plasmid was amplified by *E. coli* receptor cells, the purified plasmid was divided and sent for examination, and the lentiviral solution containing the knockdown HOXA5 vector plasmid was obtained by encapsulating the lentiviral solution with 293T cells after the sequencing was qualified for the subsequent experiments. Finally, it was concluded that HOXA5 was highly expressed in AML cells, and knockdown of HOXA5 could significantly affect the proliferation of AML cells. This experiment applied CRISPR-Cas9 technology to AML suspension cells for testing, which is a direction that has been less applied before.

CRISPR/Cas9 gene editing was applied to induce MLL-AF9 (MA9) chromosomal rearrangements in mouse hematopoietic stem. Effective ab initio generation of MA9 fusion products at the DNA and RNA levels in large populations was demonstrated by targeting the breakpoint cluster regions of mouse Mll and Af9, equivalent to those in human MA9 rearrangements. This method provides a fast and robust way to generate Mll-Af9 gene rearrangements from scratch in mouse hematopoietic stem and progenitor cells (HSPCs) [13]. MLL rearrangement (MLLr) mixed phenotype acute leukemia (MPAL) is more similar to AML than to acute lymphoblastic leukemia (ALL). Therefore, efficient generation of MLL chromosomal translocations in primary human blood stem cells using CRISPR/Cas9 can reliably mimic human acute MLLr leukemia and provide an experimental platform for basic and translational research in leukemia biology and therapeutics [14].

CRISPR/Cas9 gene editing can be also used to shear a gene in a pathogenic cell line to explore whether the gene contributes to the etiology of leukemia. They edited a cell line with the DNMT3A mutation, the DNA extraction product of which can be used as internal quality control and external quality evaluation material for AML single-gene testing, and the mutation can be stably inherited [6].

The next report depicts in detail the application of CRISPPR-Cas9 technology to target primary chronic lymphocytic leukemia and condyloma cells. This experiment assessed electroporation efficiency by labeling gRNA with a fluorescent tracer. By using this method, more than 90% of the cells contained fluorescent tracers and high transfection efficiency was successfully achieved. Next, experiments were performed to measure the percentage of CD19 alleles with insertion/deletion mutations induced by Cas9-gRNA by amplicon sequencing, and average allelic editing of 64% and 65% was observed in CLL and MCL, respectively, with no change in the percentage of allelic editing detected until the end. The project team therefore developed a method to transiently transfect Cas9-gRNA complexes into patient-derived malignant B cells. This method does not induce cell death and is highly efficient for gene editing and protein depletion [15].

A large proportion of patients either have primary resistance or develop resistance after a long period of resistance. This emphasizes the need for new treatment strategies and stratification of patient-specific therapeutic approaches. Therefore, a functional genetic CRISPR-Cas9 knockout screen was used to identify kinases ibrutinib and vinotec that may be involved in resistance. To counteract ibrutinib resistance, the study developed an adhesion loss CRISPR-Cas9 screen for the identification of several novel proteins that may be involved in BCR control of adhesion, such as PAK2 and CRKL. The presence of complex karyotypes was identified as a poor prognostic feature of vinotec therapy by analyzing the karyotypes of 895 first-treatment CLL patients. These findings open up new ideas for overcoming resistance to targeted therapies in patients with MCL and/or CLL to better their prognosis [16].

3. Conclusion

CRISPR gene editing technology in the treatment of leukemia is mainly used to knock out specific genes to achieve the inhibition of cell proliferation, but in fact, leukemia is a multi-gene changes in the results of multiple factors, only a single gene addition and deletion cannot be a complete cure for leukemia. However, in fact, leukemia is the result of multiple genes and factors, and only single gene deletion cannot completely cure leukemia.

Currently, there are two ways to apply CRISPR technology to influence leukemia treatment. The first is to knock out a known disease-causing gene, so as to inhibit the proliferation of harmful cells, thereby delaying the disease and realizing the purpose of treating leukemia. The other is to indirectly inhibit leukemia through the insertion of a certain gene by CRISPR gene editing technology to achieve the inhibition of the expression of disease-causing genes, for example, the introduction of corresponding nucleases to inhibit the transcription of cancerous genes, or the insertion of transcription termination gene sequences to terminate transcription. The other is to indirectly inhibit leukemia by inserting a certain gene through CRISPR gene editing technology to inhibit the expression of disease-causing genes, such as importing the corresponding nuclease to inhibit the transcription of oncogenic genes, or inserting the sequence of the termination gene into the disease-causing genes to terminate the transcription. There are, of course, many other related studies, such as knocking out an unknown gene in a disease-causing cell line by CRISPR gene editing to find out if that gene contributes to the cause of leukemia.

CRISPR gene editing technology in the treatment of leukemia is mainly used to knock out specific genes to achieve the inhibition of cell proliferation, but in fact, leukemia is a multi-gene changes in the results of multiple factors, only a single gene addition and deletion cannot be a complete cure for leukemia. And at present, there are fewer studies in the direction of exploring the pathogenesis and causative genes of leukemia using CRISPR technology. In the future, more researches and

experiments can be conducted to explore the pathogenesis and causative genes of leukemia using CRISPR technology.

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