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Applications of the CRISPR-Cas9 system in cancer models

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Abstract. Cancer has a high mortality and prevalence rate in the world. CRISPR-Cas9 is one of the novel and most common gene-editing techniques. Compared with the first two generations of gene-editing technologies, CRISPR-Cas9 system has the advantages of easy design, low cost, high efficiency and so on. sgRNA guides Cas9 to the site of the targeted gene, and Cas9 cuts the DNA strand at that site, triggering the NHEJ or HDR mechanism so as to achieve the purpose of deletion or insertion. CRISPR-Cas9 can be combined with other factors for other purposes, such as CRISPRa, CRISPRi, and base editing. The CRISPR system now has been used extensively for research into biological mechanisms and disease treatments. Since cancer is controlled by genes, a number of researchers in recent years have looked at using the CRISPR system to treat cancer. The CRISPR technology has greatly improved our understanding of cancer and the factors that affect it, and has had a major impact on the study and treatment of cancer. CRISPR gene editing can quickly and efficiently generate gene knockouts and regulate gene expression to identify relevant genes that influence cancer growth. This review systematically introduces CRISPR-Cas9 and its application methods, delivery modes, and discusses some studies using cell lines and organoids in vitro and animal models for cancer therapy in vivo.

Keywords: CRISPR-Cas9, cancer, therapy, model.

1. Introduction

Cancer is a common type of malignant tumor derived from epithelial tissue. Cancer cells devour healthy cells and evade the immune system, tricking it into helping them survive and grow. Cancer cells cause organ failure by pressing, squeezing, consuming, or destroying organs, which leads to abnormal functioning and mechanisms in the human body. Cancer can occur in almost any part of the body. There are cancerous tumors called malignant and not-cancerous tumors called benign. Cancer can diffuse to a new part of the body to achieve metastasis, which is a transfer of position via the lymphatic system or blood flow.

Common clinical manifestations of cancer include masses, pain, ulcers, bleeding, obstruction, and some neurological symptoms. There are many factors affecting the etiology of cancer, including exogenous factors such as lifestyle, environment, biological factors, irritations, and trauma, as well as endogenous factors such as endocrine factors and most genetic factors, which is the least common factor. The World Health Organization published a 2020 report on the cancer population in both sexes and all ages. In Asia, the death rate from cancer is 58.3 percent [1]. Clustering regular interspersed short palindromic repeats (CRISPR) is a molecular biology technique. CRISPR-Cas9 is based on an acquired prokaryotic immune system that can edit genes in cells and organisms. In this system,cas9 protein,

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sgRNA and other components are very important. There are many variants of Cas9, each type has its own set of characteristics and sequence recognition criteria, providing more flexibility to adapt as a research or therapeutic tool. As one of the most popular gene editing techniques, the CRISPR-Cas9 system can edit, activate, and inhibit the targeted gene. CRISPR-Cas9 is now used extensively for research into biological systems and treatments for human diseases [2]. This review offers an expanded description of the applications of the CRISPR-Cas9 system in cancer models.

2. CRISPR-Cas9 system

CRISPR is a kind of natural immune system of prokaryotes with a highly repetitive genetic sequence that currently protects them from foreign DNA and phages. When some bacteria were invaded by a virus, they were able to integrate a small piece of viral gene into the crisper spacer region. The crRNA precursor (pre-crRNA) is modified and processed to form a guide RNA (gRNA). When the virus strikes again, the bacteria can use this CRISPR system's gRNA for recognition by base pairing and target the Cas protein to viral DNA and cut it, rendering it useless [3]. By comparing with the first two generations of genome editing techniques, such as ZFN and TALENS, the CRISPR-Cas9 system may have a higher success rate and lower cytotoxicity. It may cost less due to the easy design and amenities for high-throughput screens. The CRISPR-Cas9 system also allows editing to occur simultaneously on different gene target sites. However, CRISPR-Cas9 technology has a lower specificity (22 bp) compared to ZFN, which has a target site length of 18–36 bp, and TALENs, which has a much longer target site length of 30–40 bp.

The CRISPR-Cas9 system is a genome engineering technology that can be used in many models to change the sequence or expression of genes. This gRNA consists of a CRISPR RNA (crRNA) sequence that binds to the target site and a tracRNA that binds to the Cas9 protein. The guide sequence in gRNA has 20 bp. The combination of crRNA and tracRNA formed a single guide RNA (sgRNA) [4]. The Cas9 protein will be guided by the sgRNA, which has the sequence of the target region of the gene, to bind to the target site. The double-strand break created by Cas9 nuclease causes a frameshift in the nucleotide sequence, inactivating the specific protein. Cas proteins require short sequence motifs, protospacer adjacent motifs (PAM sequence), to identify target sites [5]. After the Cas9-sgRNA complex combines with one of the DNA strand by identifying the PAM sequence on the complementary strand, the surrounding DNA is pulled apart to form two single-stranded DNA strands in an R-loop. The complementary strand will be clipped by the RuvC1 domain on cas9, and the complementary strand will be clipped by the HNH domain in cas9, forming a double-stranded DNA break (DSB). When cells recognize this DSB, they turn on two pathways for recovering the DSB: non-homologous end-joining (NHEJ) and homology-directed repair (HDR). NHEJ, which is also the knockout system, will produce the error-prone indel mutation, causing the frameshift and the failure of DNA. HDR, which is also called the knock-in system, requires a template strand of DNA. Double-stranded DNA or single-stranded DNA will repair itself by using another strand of DNA as a template, achieving precise gene editing.

Fusing Cas nickase (nCas) or catalytically inactive Cas nucleuses protein (dCas9) with nucleoside delaminate enzyme can produce the base editor and prime editor, inducing single nucleotide site changes without generating DSB in the target sequence or requiring repair templates. There are two types of base editors (BE), cytosine base editors (CBEs) and adenine base editors (ABEs), that can be used. CBEs carry a cytosine deaminase and cause C-to-T on the target sequence. In ABEs, the combination of adenosine deaminase and Cas9 protein induces the conversion of A-to-G transition by catalyzing the oxidative deamination of deoxyadenosine to deoxyinosine [6]. Combining transcriptional repressor or activator domains with deactivated Cas9 (dCas9), which has both cleavage domains, RuvC and HNH domains, inactivated, enables CRISPR-mediated transcriptional activation [7]. Having multiple binding sites in the same promoter can increase CRISPR activation. Multiple transcription factors and cofactors react together to stimulate a higher level of gene transcription. The transcriptional activator that is recruited by dCas9 is commonly VP64. The most effective effector is the CRISPR Synergistic Activation Mediator (SAM) complex, which may recruit MS2, P65, and HSF1 by conbining MS2 with the loop of sgRNA [8]. Such systems are available in the stimulation of single or series gene transcription. The

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binding of a single dCas9 to a promoter of target site can interfere with the binding of transcriptase in space and play a role in inhibiting the transcription of target genes. This process is called CRISPR interference. Fusing dCas9 with the Kruppel-associated box (KRAB) of Kox1, which is a transcriptional repressor or domain, allows the most effective repression of genes [9].

3. Models: disease model and therapy

3.1. In vitro

CRISPR can be applied to cell lines and organoids outside the body. Since the establishment of the first cell line (the HeLa cell line), cell lines have been used more to participate in research [10]. The use of in vitro model systems of cancer cell lines facilitates the study of biological processes and improves outcomes and therapy. Using CRISPR knockin and knockout, we can study the function of genes that affect cancer cells. Using CRISPRa and CRISPRi, you can change the expression level of related genes in cancer cell lines. Compared to animal models, the use of cancer cell lines is less costly and easier to maintain and store. The result is high productivity, and cancer cell lines are amenable to CRISPR.

One important application is to use the CRISPR-Cas9 system to study the relationship between genetic mutations and drug resistance in cancer cells. Drug resistance in cancer cells reduces the effectiveness of drugs and becomes an obstacle to cancer treatment. For example, a knockout in one study using the CRISPR-Cas9 system in the ABCB1 gene (also known as the MDR1 gene) in cancer cells could increase the sensitivity of chemotherapy as well as the concentration of specific chemotherapy drugs in cancer cells [11]. Another study used the CRISPR system with the lung cancer cell line to show a relationship between the NRF2 gene in lung cancer cells, whose upregulation leads to increased drug resistance, and chemotherapy drugs, including Cisplatin [12]. When lung cancer cells are treated with chemotherapy drugs, the volume and proliferation rate of cancer cells are reduced by CRISPR knockout, which means that cancer cells become more sensitive to chemotherapy drugs. CRISPR-Cas9 can create mutations in cancer cell lines in order to study drugs and resistance to that cancer. In one study, the CRISPR system was applied to introduce epidermal growth factor receptor (EGFR) T790M mutations into the PC9 human lung cancer cell line [13]. It was found that the mutation of T790M in EGFR T790M PC9 cells produced by CRISPR-Cas9 is higher than in PC9 cells produced by gefitinib with the same mutation (PC9-g) after long-term exposure. CRISPR could also be used to screen and probe oncogenes for specific cancers [14].

Organoids were gradually built, transforming the 2D medium into the 3D medium. Organoids, which are organ-specific three-dimensional cell clusters, bridge the gap between in vitro model system cell lines or primary cells and in vivo studies because the organoids built up rom adult stem cells are very similar to the tissues of their origin [15]. Because of the heterogeneity and histological features of the primary tumor, PCDOs are an ideal model for the study of anticancer drugs. Organoids can partially simulate the process of cancer and its treatment. Because mouse models differ significantly from humans, the use of patient-derived cancer organoids (PCDOs) would be more precise, and the results of the study could be applied to human bodies. Due to the heterogeneity and histological characteristics of the primary tumor, PCDOs is a suitable model for the study of anticancer drugs [16]. Multiple cancer organoids have been grown for further study of each cancer. It has been demonstrated that induction of NHEJ using CRISPR-Cas9 technology can introduce the required mutations to transform normal organoids and induce tumorigenic growth in xenografts to simulate tumorigenesis [17]. The study targeted genes such as KRAS, APC and p53. Mutations in the KRAS gene, which is an oncogene, are commonly found in colon cancer. This specific genomic alteration is introduced into the KRAS gene of the organoid through HDR-directed DNA repair to establish an organoid model of colon cancer occurrence. Also, researchers can expand and grow primary cancer organoids from cancer patient tissue. The result is an organoid model of cancer that could help researchers use patient tissue directly for targeted studies and rapid and cost-effective personalized drug testing. For example, one study determined the mechanism of oxaliplatin resistance in peritoneal metastases (PM) in colorectal cancer (CRC) and studied strategies to overcome this resistance [18]. They demonstrated that targeting drugs

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using oxaliplatin in combination with redox-targeting drugs have a higher anticancer effect in patients with colorectal cancer by collecting ten stable sources of PM from six patients to establish organoid techniques for studying resistance. CRISPR-Cas9 is often used in cancer organoids to induce NHEJ and HDR mechanisms to disable targeted genes, such as oncogenes, genes that contribute to cancer growth and spread, in order to screen for genes affecting cancer or to develop treatments for cancer.

3.2. Delivery system in vitro

In vitro cell lines, the CRISPR system is typically delivered via microinjection, lentiviral vectors, or electroporation. Mutations in tumor suppressor genes, such as TP53 [19], and uncontrolled expression of oncogenes, such as KRAS [20], in cell lines lead to cancer. Viral vectors, including lentivirus (LV), adenovirus (AdV), and adeno-associated virus (AAV), are commonly used for transport in the CRISPR-Cas9 system in organoids. Viral vectors have significant advantages over other vectors in terms of transport efficiency and tissue specificity. In recent years, because of its unique advantages, AAV vector has been widely used in the development of gene therapy products, and has great potential [21].

3.3. In vivo

CRISPR-Cas9 can also target tumors directly in vivo, which is harder and more challenging. Mouse models are usually used. CRISPR screens for the gene to relate to a specific cancer or test the feasibility of the drug by using CRISPR-Cas9 are two common uses for the application in vivo. In a study, Meiou Dai et al. used the CRISPR system to reveal the vulnerabilities of breast cancer and establish an innovative therapeutic approach through the pharmacological inhibition of torin1-mediated mTORC1/2 and the oncoprotein YAP [22].

Tumor cells are first injected into animal models, usually mice with immune deficiencies. And allow the tumor to develop over time to create an animal model of cancer that can be observed. Injecting the CRISPR system into its animal model, for example by using a viral vector. By knocking out cancer-causing genes, the genes lose their function and thus hinder the development of cancer. Finally, the tumor volume can be measured to determine the feasibility of a treatment or it can be screened for genes that affect the cancer of interest by using simultaneous multi-site targeting genes. A mislink between pieces of DNA from two different genes can lead to a fusion gene, which is an accident that occurs during cell division. They can turn fusion genes and the proteins they encode into factors that trigger tumor formation and are called fusion oncogenes. Fusion oncogenes (FOs) occur in many cancer types and contribute significantly to the development of cancer. Since the elimination of FOs induces apoptosis of cancer cells, a study in 2020 proved that the elimination of FOs can help the treatment of cancer by studying the efficacy of intron-based targeting of transcription factors or tyrosine kinase FOs in reducing tumor burden and mortality in in vivo models [23]. adeno cas9 was injected into Ewing's sarcoma and chronic myeloid leukemia cell lines and mouse models using adv virus vector injection, and was cleaved by targeting FOs introns at both ends. As the cell attempts to repair the break, it joins the cut end, causing the fusion gene in the middle to be completely wiped out.

3.4. Delivery system in vivo

Different target cells will have different suitable delivery vectors. For example, when the target cells are liver cells, plasmids with Cas9 protein genes can be delivered intravenously to the liver, which should use viral vectors or other ways for higher efficiency [24]. The common delivery methods are lipid nanoparticles (LNPs) and viral vectors. LNPs, which are nanoparticles composed of lipid and typically spherical, are a material used for mRNA delivery [25]. Since mRNA has shown great therapeutic potential in many clinical trials and clinical applications, the research and improvement of LNPs will advance the treatment of more diseases. LNPs were applied to mRNA-1273, the coronavirus disease (COVID-19) vaccine approved in 2019 that uses RNA vaccine technology, as used for SARS-CoV-2 which is the virus that causes COVID-19, delivery vehicles, delivering the antibody mRNA [26]. A study conducted in 2020 used CRISPR-Cas9 technology with LNPs and AAV vectors to disrupt the ability of PLK1 gene expression to treat and study cancer [27].

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Viral vector is also a very important transmission medium. The most commonly used Crispr-Cas9 system delivers viral vectors, which are involved in the treatment of many diseases. There are adeno-associated viruses (AAVs), adenoviral vectors (AdVs), and lentiviral vectors (LVs). AAV is a small, uncoated single-stranded DNA virus that has a relatively low probability of immunogenicity, cytotoxicity, and chromosome integration, making it a typical delivery system in vivo [28]. For example, AAV was also used as a delivery vector in a study that reduced PLK1 gene expression capacity, as mentioned above in the last paragraph [27]. AdV as a double-stranded DNA virus can transduce a large number of dividing and non-dividing cells and generate episomal DNA around host DNA without integration into the genome [28]. Thus, ADV can reduce the off-target effect in genome editing. Lentiviral vectors (LVs), which are single-stranded RNA viruses, are gene therapy vectors developed based on the HIV-1, which is the human immunodeficiency virus I. LVs integrate foreign DNA into the host genome, resulting in persistent gene expression.

4. Conclusion

CRISPR-Cas9 is a relatively new gene-editing technique. Different tools added to the Crispr system can generate different functions. As CRISPR-Cas9 expands and advances, it is increasingly making significant contributions to research and treatment strategies for diseases that are inherited or directly linked to genes. It has great potential to be realized in clinical medicine. In cancer, CRISPR-Cas9 has led to a better understanding of what causes cancer and a better way for people to develop cancer drugs. However, CRISPR-Cas9 is a broad and promising technology that will continue to advance and develop more work and research remain to be done to achieve the universal use of CRISPR-Cas9 in clinical treatment. For example, CRISPR-Cas9 has been facing obstacles due to an off-target effect. It will also continue to be explored and studied in the application of cancer therapy.

References

[1] Ferlay J, et al. (2020). Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today

[2] “The Genetics of Cancer.” National Cancer Institute, 17 Aug. 2022, www.cancer.gov/about-cancer/causes-prevention/genetics.

[3] ZHENG Wu, GU Feng. Research progress on application and off-target effect of CRISPR/Cas9[J].Heredity,2015,37(10):1003-1010.

[4] Jinek, Martin, et al. “A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive. Bacterial Immunity.” Science, vol. 337, no. 6096, American Association for the Advancement of Science, 17 Aug. 2012, pp. 816–21.

[5] Gleditzsch, Daniel, et al. “PAM Identification by CRISPR-Cas Effector Complexes: Diversified. Mechanisms and Structures.” RNA Biology, vol. 16, no. 4, Taylor and Francis, 3 Apr. 2019, pp. 504–17.

[6] Tan, Jun-Jie, et al. “DNA Base Editing in Nuclear and Organellar Genomes.” Trends in Genetics, vol. 38, no. 11, Elsevier BV, 1 July 2022, pp. 1147–69.

[7] Heidersbach, et al. A versatile, high-efficiency platform for. CRISPR-based gene activation. Nat Commun 14, 902 (2023).

[8] Genomics, Sigma-Aldrich® Advanced. “Why You Should Consider Adding CRISPRa and. CRISPRi to Your Toolbox.” Bitesize Bio, 27 May 2020.

[9] Gilbert, L. A. et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell 154, 442–451 (2013). Along with Qi et al., this paper describes the use of Cas9 for targeted control of transcriptional activation and repression.

[10] Mirabelli, Peppino, et al. “Cancer Cell Lines Are Useful Model Systems for Medical Research.” Cancers, vol. 11, no. 8, MDPI, 1 Aug. 2019, p. 1098.

[11] Yang Yang, et al . “KRAS Mutation: From Undruggable to Druggable in Cancer.” Signal Transduction and Targeted. Therapy, vol. 6, no. 1, Nature Portfolio, 15 Nov. 2021,

[12] GAO Meng, ZHAI Nailiang. Role of Keap1/Nrf2 in treatment resistance of non-small cell lung cancer[J].Journal of Binzhou Medical College,2022,45(06):467-471.
[13] Wang Li. Preparation of sgRNA and CRISPR/Cas9 by overlapping primer PCR for EGFR deletion mutation enrichment in lung cancer ctDNA[D].Southern Medical University,2021.
[14] He, Chenglong. "CRISPR Screen in Cancer: Status Quo and Future Perspectives." PubMed Central (PMC), 2021,
[15] WANG Haixia, ZHANG Cong, ZOU Dongling. Application of organoid model in precision diagnosis and treatment of tumors[J].Chinese Clinical Oncology,2023,50(06):291-295.
[16] Geurts, Maarten H., and Hans Clevers. "CRISPR Engineering in Organoids for Gene Repair and Disease. Modelling." Nature Reviews Bioengineering, vol. 1, no. 1, 19 Jan. 2023, pp. 32–45..
[17] HU Kun, LIU Yuhan, XIAO Junwen, LIAO Xinhui, CHEN Jieqing,ZHANG Zhongfu,WU Jianting,MEI Hongbing. Progress of CRISPR/Cas9 gene editing technology in prostate cancer research[J].Life Science,2019,31(07):731-738.
[18] Cancer Belong to Consensus Molecular Subtype 4 and Are Sensitised to Oxaliplatin by Inhibiting Reducing. Capacity." British Journal of Cancer, vol. 126, no. 12, Nature Portfolio, 22 Feb. 2022, pp. 1824- 33.
[19] LI Yaona, YANG Huiyu. Research progress of TP53-induced glycolysis and apoptosis regulators in common cardiovascular diseases[J].China Medical Journal,2023,18(04):599-602.
[20] Huang, Lamei, et al. "KRAS Mutation: From Undruggable to Druggable in Cancer." Signal Transduction and. Targeted Therapy, vol. 6, no. 1, Nature Portfolio, 15 Nov. 2021.
[21] DENG Yichen, LIU Yunbo. Research progress of AAV vector targeted modification strategy in gene therapy[J].Chinese Journal of Comparative Medicine,2017,27(02):81-85.
[22] Dai, Meiou, et al. "In Vivo Genome-wide CRISPR Screen Reveals Breast Cancer Vulnerabilities and Synergistic. mTOR/Hippo Targeted Combination Therapy." Nature Communications, vol. 12, no. 1, Nature Portfolio, 24 May 2021.
[23] M, Martinez-Lage, et al. "In Vivo CRISPR/Cas9 Targeting of Fusion Oncogenes for Selective Elimination of. Cancer Cells." Nature Communications, vol. 11, no. 1, Nature Portfolio, 8 Oct. 2020.
[24] Thomsen, Martin K. "Application of CRISPR for in Vivo Mouse Cancer Studies." Cancers, vol. 14, no. 20, MDPI, 13 Oct. 2022, p. 5014.
[25] Hou, Xucheng, et al. "Lipid Nanoparticles for mRNA Delivery." Nature Reviews Materials, vol. 6, no. 12, Nature. Portfolio, 10 Aug. 2021, pp. 1078–94.
[26] Pardi, Norbert, et al. "mRNA Vaccines — a New Era in Vaccinology." Nature Reviews Drug Discovery, vol. 17, no. 4, Nature Portfolio, 12 Jan. 2018, pp. 261–79.
[27] Rosenblum, Daniel, et al. "CRISPR-Cas9 Genome Editing Using Targeted Lipid Nanoparticles for Cancer. Therapy." Science Advances, vol. 6, no. 47, American Association for the Advancement of Science, 18 Nov. 2020.
[28] Ahmadi, Seyed Esmaeil, et al. "Viral Vectors and Extracellular Vesicles: Innate Delivery Systems Utilized in. CRISPR/Cas-mediated Cancer Therapy." Cancer Gene Therapy, Springer Nature, 28 Feb. 2023.