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Cancer Study Using CRISPR-Cas9 Gene Editing Technology

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ABSTRACT

In the field of genetic engineering, CRISPR/Cas9, which was first identified as an adaptive immune system in bacteria, has emerged as a revolutionary tool. Recent technological developments have improved the CRISPR/Cas9 tool's utility for gene editing, gene treatments, developmental investigations, and mutational analyses in different model species. The best animal model to use for CRISPR/Cas9 research to determine the functional significance of certain genes of interest is the zebrafish. Knock-out and knock-in procedures, which are CRISPR/Cas9 mediated gene editing techniques, give evidence to discover the function of various genes through loss-of-function investigations. Additionally, based on phenotypic screening, CRISPR/Cas9 has been shown to be a successful technique for creating disease models for gene expression research. The current chapter gives a CRISPR/Cas9 overview.

Keywords: Crispr, Gene editing, RNA interference (RNAi), zinc finger nucleases (ZFNs), Transcription activator-like effector nuclease (TALENs).

INTRODUCTION

The CRISPR-Cas9 gene-editing system [1] has the ability to alter the behaviour of tumor cells at the genome level, lessen the killing of healthy human tissue cells, and prolong patient survival. This technique has been regarded as a flexible tool for genome engineering since it has a high [2] editing efficiency and little off-target effects.

The advantages of CRISPR Cas9 as a sophisticated gene editing technology.

A number of methods, including RNA interference (RNAi), zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs), have been developed to accomplish precise gene editing.

The CRISPR-Cas system, which was initially identified in Escherichia coli (such as "S. pyogenes"), is the system that has been used externally the most in mammals. There is a sin Glycine guide RNA in it.

Application of CRISPR -Cas9 in gene screening

Identification of the primary genetic dependencies and important oncogenic mutations in malignancies is crucial for understanding the molecular processes of carcinogenesis and investigating potential treatment targets. The need for effective and impartial in vitro and in vivo gene screening is therefore critical.

It has been demonstrated that maternal embryonic leucine zipper kinase (MELK) is required for the growth of certain cancer cells by RNAi [3] or small-molecule targeted medications. OTS167 is viewed as a novel therapeutic target for breast cancer due to its ability to inhibit the growth of MELK-knockout cell lines.

Functional oncogene fusion screening may now be done using CRISPR-Cas9 technology. In the clinic, the effectiveness of anticancer medications gradually declines, which is mostly attributed to cells' elevated production of drug-resistant genes. These studies support the utilization of a

Viral-based delivery system

Adeno-associated virus (AAV), lentivirus, and adeno-virus are commonly used to deliver the plasmid-based CRISPR-Cas9[4] system in cancer research. Wang G et al. successfully induced liver cancer by mutating the liver of Cre-inducible Cas9 mice with an AAV carrier. Unlike AAVs and lentiviruses, adenoviruses have larger transgene sizes and fewer sequence restrictions.

Nonviral delivery system

Nonviral delivery of CRISPR-Cas9 and piggyBac transposons can achieve transduction efficiencies of up to 92.43% in mouse [5] livers, making them safer for the host. receptor (ASGPR) is a c-type lectin expressed almost exclusively on the surface of liver cells. D r. Zemin Chen et al. developed liposome-templated hydrogel nanoparticles (LHNPs) combined with minicircle DNA techycratology to deliver a system targeting Plk1, which repressed Tumor growth and improved survival rates.

Application of CRISPR -Cas9 in cancer models

CRISPR-Cas9 is being used to create models of cancer that are similar in behaviour to that of a tumor and can simulate the mutation specualtrum observed during the occurrence and development of cancer.[6]

Hepatocellular carcinoma

Researchers are exploring the possibility of triggering multiple mutations in mouse liver cells using CRISPR/Cas9 to induce human hepatic cell death (HCC) - a form of cancer that can be induced by targeting specific genes. For example, Wen Xue et al. constructed a mouse liver[7] cancer model by targeting the tumor suppressor genes P ten and p53 in the mouse liver.

Pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is a highly malignant cancer with a poor prognosis. Pancreatic cancer is expected to be the second leading cause of cancer-related death by 2030[8]. To understand the underlying processes of pancreatic cancer, various pancreatic cancer models have been constructed.

Colorectal cancer

Colorectal cancer can be accurately molded by knocking out MLH1 using the CRISPR-Cas9 technique. In situ gene editing was successful in wild-type mice using lentiviral constructs expressing a short guide RNA targeting APC and Cas9.

Lung cancer

Lung Tumors can be induced in mice through CRISPR-mediated gene mutations and chromosome rearrangement. Platt RJ et al. used the Cas9 gene to deliver sgRNAs through an AAV vector to mice, causing functional deletion mutations of p53 and Lkb1 in the lung.

Breast cancer

The CRISPR -Cas9 system has been used to generate a variety of breast cancer models for study. In situ induction of gene expression in mice can mimic that in humans. Organoid transplants of breast cells into mice can also mimic human tumor production in mice.

Application of CRISPR -Cas9 in cancer treatment

Researchers are investigating its potential as a cancer therapy that targets certain cancer genome sequences or driving mutations linked to cancer growth.

Using CRISPR, Chen Z Hetal. discovered the distinct sequence created by the TMEM135-CCDC67 or MAN2A1-FER gene rearrangement in the genome of cancer cells. In mice models of lung cancer, the Cas9 method and the herpes simplex virus type 1 thymidine kinase (HSV1-tk) gene were inserted. In mouse xenograft models, the tumor load was shown to have reduced, and no animals perished during the research. Although most tumors are inactive in response to CAR-T cell treatment, it is effective in treating B cell acute lymphoblastic leukemia and chronic lymphoblastic leukemia. CRISPR-Cas9 technology can be used to genetically alter CAR-T cells to boost their anticancer activity.

Non-coding areas can be targeted by CRISPR-Cas9 methods as

Limitations and future directions CRISPR-Cas9: Of-target effects and immune response

The CRISPR-Cas9 gene-editing technologies of target effects impede its clinical application. Jinek et al. found that the seed sequence based on the 3'-end of the sgRNA sequence, adjacent to the upstream PAM, had a low tolerance for base mismatches in Cas9 endonuclease. New techniques have been developed to sensitively detect and identify new sites for cleavage by sequencing

Researchers are trying to reduce the number of potentials of-target effects of genomic CRISPR-Cas9 nuclease for use in gene therapy. CIRCLE-sequencing, a high-throughput analysis of Cas9 genome-wide activity, has been developed by Pinar Akcakaya and colleagues at the University of Texas A&M in the US [12].

To overcome the immunogenicity of SpCas9, Ajina R et al. crossed Rosa26-LSL-Cas9 knocking (Cas9- KI+/+) male mice in the C57BL/6 J background with WT female mice to obtain heterozygous Cas9 transgenic mice. Multicolour flow cytometry analysis found no significant difference in the spleen immune population between the two groups [13].

Conclusion

CRISPR-Cas9 gene-editing technology has broad application prospects in cancer research and can lead to the development of effective strategies and improve research. In addition to the of-target effects and the immune response described above, ethical issues are also of concern for this technique, which have long been debated.

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