Mini Review
Volume 6:5, 2022

Journal of Genetics and DNA Research

ISSN: 2684-6039 Open Access

CRISPR/Cas9 Gene Editing in Cancer: Current Applications and Future Prospects

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Abstract

In prokaryotes, the clustered regularly interspaced short palindromic repeats (CRISPR) system provides adaptive immunity against plasmids and phages. This system inspired the development of the CRISPR/Cas9 genome editing system, which is a powerful genome engineering tool. The CRISPR/Cas9 technique has been used to investigate the functions of cancer-related genes, establish tumor-bearing animal models and probe drug targets, greatly increasing our understanding of cancer genomics. In this paper, we examine the current state of CRISPR/Cas9 gene editing technology in oncological research. We begin by explaining the fundamental principles of CRISPR/Cas9 gene editing before introducing several new CRISPR-based gene editing modes. The rapid progress of CRISPR screening in revealing tumorigenesis, metastasis and drug resistance mechanisms is then described.

Keywords: CRISPR/Cas9 • Gene editing • CRISPR screen • Gene delivery

Introduction

Great inventions and discoveries are frequently told as a series of fortunate coincidences. A closer examination of their history, however, reveals that truly serendipitous discoveries are extremely rare, if not non-existent, in molecular biology. This may be true for other scientific disciplines as well. There are several characteristics that distinguish groundbreaking scientific advancements. They are frequently the result of decades of collaboration between many brilliant minds. Even so-called serendipitous discoveries occur when an inquisitive and open-minded researcher devises a series of meticulous experiments in response to an intriguing observation [1]. During this process, researchers with creative minds and extensive background knowledge can seize the opportunity to bring seemingly disparate research fields together and make a greater scientific impact.

Description

Breakage of both DNA strands endangers genome stability. Eukaryotic cells can repair this fatal damage in two ways: non-homologous end joining (NHEJ) and homology directed repair (HDR) (HDR). NHEJ is a clumsy repair mechanism that simply connects broken ends, resulting in random insertions or deletions (indels). This may result in frameshift mutations and, as a result, gene function loss. In contrast to NHEJ, HDR reconstructs broken DNA using homologous DNA templates. As a result, by generating DSBs at specific sites in the genome and introducing a donor template into the target cell, it is theoretically possible to precisely modify the genome sequence. Identifying the potential causes of psychological anguish and then resolving to take efforts to reduce or overcome it is the first stage in effective dealing with psychological distress. This may entail psychiatric counselling in order to identify the source of the psychological suffering. A psychiatrist, psychologist, or other mental health practitioner may recommend a variety of therapeutic treatments to assist relieve psychological discomfort as part of the counselling [2,3].

Epigenetics is the study of inherited changes in gene expression that do not involve changes in DNA sequence. DNA methylation and demethylation, histone posttranslational modifications, chromatin remodelling and non-coding RNA changes are all epigenetic mechanisms that play important roles in a variety of biological processes. To carry out CRISPR/Cas9-mediated epigenome editing, a nuclease-dead Cas9 (dCas9) is fused with a transcription activator or repressor domain, known as an epigenetic effector (epieffector). DCas9 is a DNA-binding domain with no nuclease activity. Many studies have concluded that the dCas9-epieffector fusion complex is a useful tool for epigenome editing. The Krüppel-associated box (KRAB) effector domain was used in one in vitro study [4,5].

Conclusion

The CRISPR/Cas9 system derived from Streptococcus pyogenes was used in the majority of studies (Spy Cas9). SpyCas9 gRNAs typically have a 20-nt guide sequence with a PAM requirement of 5′-NGG-3′. The development and optimization of the CRISPR/Cas9 genome editing reagent and delivery method over the years has significantly improved the safety and efficiency of gene editing in HSPCs. Early attempts for Cas9 and gRNA expression used a plasmid DNA-based system, which resulted in low editing efficiency and high toxicity. However, achieving high editing efficiency must be balanced against potential safety concerns about off-target mutations and immunogenicity caused by sustained or excessive expression of CRISPR components. Although all components of the editing machinery elicited immune, stress and apoptotic responses, delivering gRNA and Cas9 as a pre-complexed ribonucleoprotein (RNP) is well tolerated in mice.

Acknowledgement

None.

Conflict of Interest

There are no conflicts of interest by author.

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Date of Submission: 03 September, 2022, Manuscript No. jgdr-22-77735; **Editor Assigned:** 06 September, 2022, PreQC No. P-77735; **Reviewed:** 18 September, 2022, QC No. Q-77735; **Revised:** 22 September, 2022, Manuscript No. R-77735; **Published:** 27 September, 2022, DOI: 10.37421/2684-6039.2022.6.134

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How to cite this article: Ventura, Mario. "CRISPR/Cas9 Gene Editing in Cancer: Current Applications and Future Prospects." J Genet DNA Res 6 (2022): 134.