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Review

CRISPR-Cas Associated Cells and Animal Mediated Biomedical Modelling

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Abstract: Gene editing is now easy to make new disease models through in-vivo and in vitro tests. It can potentially be used to make animals with single-gene or multiple-gene changes. The mutant strains with changed germlines are no longer needed with in vivo gene editing, which uses the CRISPR-Cas9 system to target cells of interest in their normal tissues. Whereas, the AAVs and other viral vectors have made it possible to change cells selectively. Gene editing hade made it possible to use human induced pluripotent stem cells (iPSC) to model diseases that run in families. Researchers can compare and contrast the human genomes of many different ethnic and racial groups using this method. Scientists may be able to make a disease in a lab dish using iPSCs from a patient. Using CRISPR, iPSCs made from patient cells can be fixed if they have certain problems. This shows that gene therapy is possible and shows what happens when cells aren't working right. The fact that CRISPR-Cas9 can change the DNA by just one nucleotide has had a huge effect on biological studies. CRISPR is becoming more and more popular, which shows how useful, easy, and effective it is. With the broad use of CRISPR-based apps, the tool is now used for much more than just changing genes. This method can be used to screen the whole genome, control the translation of genes based on their sequence, and edit several genes at the same time. Scientists can now model diseases in different species and learn more about how genes work because of these advances. Genome-wide association studies and genome-editing tools like CRISPR are giving us a good look at the future of personalized medicine.

Keywords: CRISPR-Cas system; genome engineering; biomedicine; human disease; animal models

Introduction

CRISPR gene editing is now easy to make new disease models quickly through in vivo and in vitro tests. Some of the most recent choices are putting sgRNAs and Cas9 mRNA into eggs with just one cell to change their DNA. Rodent, rat, and monkey models have all been made with CRISPR-Cas9. [2-5] This shows how quickly it can be used to make animals with single-gene or multiple-gene changes. Additionally, the mutant strains with changed germlines are no longer needed with in vivo gene editing, which uses the CRISPR-Cas9 system to target cells of interest in their normal tissues. This method is used for gene therapy because it can be used with both disease models that already exist and modified breeds. Whereas, the AAVs and other viral vectors have made it possible to change cells selectively. Gene editing hade made it possible to use human induced pluripotent stem cells (iPSC) to model diseases that run in families. Researchers can compare and contrast the human genomes of many different ethnic and racial groups using this method. Scientists may be able to make a disease in a lab dish using iPSCs from a patient. [1] Using CRISPR, iPSCs made from patient cells can be fixed if they have certain problems. This shows that gene therapy is possible and shows what happens when cells aren't working right. [4]

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Biomedical Modelling

Researchers have turned to CRISPR models to learn more about the molecular processes that cause cancer, brain diseases, and other Mendelian or difficult genetic diseases in people. Also, these models could be used to quickly test a number of potential drugs and gene therapies. [2,3,6]

Cancer Modelling

Cancer is good example of CRISPR-Cas associated cells and animal mediated biomedical modelling. In-vivo and in vitro modelling for cancer therapy take a lot of effort and work. Transfection-based multiplex transfer method used to send genetic parts by CRISPR-Cas system into the pancreas of adult mice. This changed a lot of gene network sets, which led to the animals getting pancreatic cancer in the end. [3-4] In the same study, models were made to show the complicated changes to chromosomes that can be used to diagnose pancreatic cancer. Investigation make it possible to looked into genes in mouse models of cancer in 2014. A lung cancer model that was driven by Kras (G12D) showed this. CRISPR-Cas9 was used to change the DNA sequence of tumor-suppressing genes in lung cancer cases in which the genes had stopped working. Because of this, the animals got lung adenocarcinomas. [7,10]

Brain and Spinal Cord Models of Illnesses

Several high-quality studies explain in detail how these models work. Different studies conducted to make iPSC-based model to find out how changing the genome with CRISPR-Cas9 can cause seizures in SCN1A. In the modeling process, iPSCs from both the patient and the control group were used. Engineered iPSCs were used to make GABAergic neurons, which were then fluorescently tagged and found using the "knock-in" method. Using this method, scientists found that Nav1.1 was mostly expressed on GABAergic neurons and very rarely on glutamatergic neurons during this time of brain development. Tabebordbar et al. used CRISPR in vivo to try to fix the abnormalities that cause Duchenne muscular dystrophy (DMD) in the skeletal muscle and muscle stem cells of mice. All of these things helped people with Duchenne muscular dystrophy. By using AAVs to send Cas9 and sgRNAs to both ends of exon23, the mutant Dmd gene's exon23 is taken out. Through this process, a shorter protein with the same purpose is made. Things did get better, but only to a certain degree.[8,9,11]

Use of Virtual reality to Mimic Heart Disease

Virtual reality used to mimic heart disease for investigation and diagnosis. Targeting of the Cas9 gene at particular site of the heart has recently tailed to make an animal model. Whereas, the Cas9 translation plasmid controlled by the Myh6 promoter is put into a mouse zygote, Cas9 is only made in the cardiomyocytes of the heart. Researchers showed that AAV can be used to send sgRNAs to the heart that turn off the Myh6 gene. In 2014, adenovirus used to send sgRNAs that target Cas9 and Pcsk9 to the liver of mice. This caused certain changes in the original Pcsk9 gene that made it lose its ability to work. The writers looked into what the shockingly high rate of change (about 50%) would mean for how the disease would get worse. Researchers saw that the amount of fat in the blood of the animals went down.[13-15]

GFP Tagged Cas9 Lentivirus Cells Infection

CRISPR-Cas9 parts tagged Green Fluorescence Protein (GFP) infected the cells by lentivirus reporter in both pre-integration viral genomes and integrated proviruses. With CRISPR, which gets rid of only latently infected T-cell lines and the cells that house HIV (monocytes and macrophages), long-term defense against HIV-1 has also been achieved. The genome-editing tool CRISPR-Cas9 may stop viral genes from being made and copied by focusing on and cutting off conserved parts of the genome of the chronic hepatitis B virus.[15,17]

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Immuno-deficient Animal model

Immunodeficiency can be seen in animals. CRISPR can be used to make a wide range of animals without immune systems. Cas9 mRNA and a set of sgRNAs were microinjected into growing mice to target the B2m, Il2rg, Prf1, Prkdc, and Rag1 mouse genes. [18,19]

Table 1. Overview of the use of the CRISPR-Cas9 systesm in the context of disease modelling.

Disease	CRISPR approach
Cancer	Hematopoietic stem and progenitor cells from a mouse without MLL3
Cancer	An in vivo liver model was used to make a -catenin activating point mutation happen again, and Pten and p53 were knocked out.
Cancer	The t(11;22) and t(8;21) translocations are replicable in human HEK293, mesenchymal, and hematopoietic cells.
Cancer	An in vivo lung model using the NIH/3T3 cell line for studying chromosome inversion and p21p23 induction
Cancer	In a Kras(G12D)-driven lung cancer model, many tumor suppressor genes are inactivated.
Cancer	Treatment of a patient-derived colon cancer cell line by reversing a mutation in a protein kinase C (PKC)
Cancer	Restoration of PKC function in a patient-derived colon cancer cell line
Cancer	Translocation t(2;13)(q36.1;q14.1) in human alveolar rhabdomyosarcoma is replicated in mice myoblast cells.
Cancer	Loss-of-function screening at high throughput for detecting drivers of lung metastasis and growth in non-small-cell lung cancer
Cancer	Loss-of-function screening at high throughput for identifying regulators of NSCLC progression and metastasis to the lungs
Cancer	High-throughput screening for loss-of-function mutations in non-small-cell lung cancer
Cancer	high-throughput loss-of-function screening for preventing the spread of non-small-cell lung cancer to the lungs
Cancer	Pancreatic Lkb1 deletion in an in vivo model.
Cancer	Knockdown of TP53 in an in vitro model of oesophageal adenocarcinoma
Cancer	Somatic multiplex mutagenesis as a tool for high-throughput mouse gene function investigation
Cancer	In vitro model of exon 14 deletion utilizing the HEK293 cell line
Cancer	Systematic knockdown of the TP53 gene was performed on HCT116 colorectal and H460 lung cancer cells in an in vitro model.
Cancer	Deletion of JunB in a cultured model of head and neck squamous cell carcinoma
Cancer	Knocking down NoxO1 in a human colon cancer cell culture model
Cancer	Method for reprogramming human T cells using a PD-1 knockout model
Cancer	Knocking off PYCR1 in a mouse model of invasive breast cancer
Neurological	DMD exon 45-55 deletion in human iPSCs
Neurological	Duchenne muscular dystrophy (DMD) exon 23 deletion was achieved in an MDX mice model of the disease by delivering AAV9 intraperitoneally, intramuscularly, or retroorbitally.
Neurological	DMD exon 23 deletion mouse model for Duchenne muscular dystrophy
Neurological	DMD exon 23 deletion is seen in the tibialis anterior muscles of a mouse model of Duchenne muscular dystrophy.
Neurological	Pmm2 knockout in a Drosophila embryonic stem cell model
Neurological	Fluorescent labeling of iPSC-derived GABAergic neurons
Neurological	Tenm1-deficient (knockout) mice
Cadiovascular	Pcsk9 deletion using adeno-associated virus in a living mouse liver model
Cadiovascular	Cardiac-specific Cas9 transgenic mice and Myh6 knockout cells are generated after AAV9 delivery in cardiomyocytes.
Infectious	The suppression of HBV viral gene expression and replication by the selective targeting and cleavage of conserved regions of the HBV genome.

[1,2,4, 10, 12, 16, 20-23.]

Conclusion

The fact that CRISPR-Cas9 can change the DNA by just one nucleotide has had a huge effect on biological studies. CRISPR is becoming more and more popular, which shows how useful, easy, and effective it is. With the broad use of CRISPR-based apps, the tool is now used for much more than just changing genes. This method can be used to screen the whole genome, control the translation of genes based on their sequence, and edit several genes at the same time. Scientists can now model diseases in different species and learn more about how genes work because of these advances.

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Genome-wide association studies and genome-editing tools like CRISPR are giving us a good look at the future of personalized medicine.

Authors' contributions and materials: This work was carried out in collaboration among all authors. **Taha Nazir** designed the study of proposed hypothesis and compile the scientific contents. **Nida Taha** elaborated study to make it more credible. Whereas, **Hameed A Mirza** managed the literature searches and citation part of the manuscript. Thus, all authors have read and approved the final manuscript for publication in this journal.

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Animal rights: Additionally, this research studies no animals involved. The authors indicate the procedures followed are in accordance with the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals; published by the National Academy of Sciences, The National Academies Press, Washington, D.C.).

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