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Gene-edited animal models in human diseases: A powerful tool

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Abstract. With the rapid development of gene editing technology, gene editing and the use of animal models have become an integral part of biomedical research. Gene editing has had a disruptive impact in the life sciences, having evolved from the first generation of ZFN, the second generation of TALEN to the current CRISPR. The advent of gene editing technology has greatly increased the possibility of treating hereditary diseases and cancers. Animal models make a key contribution to research into disease research as they provide scientists with a valuable platform for the in-depth understanding and treatment of human diseases. Through animal models, scientists can simulate human diseases and study the pathogenesis, pathological processes and potential treatments of human diseases, thus securing the unique platform provided. This article systematically reviews the application of gene-edited animal models in the study of neurodegenerative diseases, cancer and metabolic diseases, and discuss their important role in advancing disease research.

Keywords: Human diseases, gene-editing, animal models.

1. Introduction

Animal models make a key contribution to research into disease research as they provide scientists with a valuable platform for the in-depth understanding and treatment of human diseases. Firstly, scientists are able to study the pathogenesis of disease by observing and experimenting on animal models to simulate the development of disease. Secondly, scientists are able to try out newly developed drugs on animal models to test their safety and toxicity, thereby screening out drug candidates, reducing the risk of later clinical testing and improving the success rate of drug development. It can also provide feedback on the vaccines being tested, which can inform the optimisation and design of the vaccine. Finally, certain features of human diseases can be replicated by animal models, especially in the field of genetic and metabolic diseases, which offer important tools for studying the pathogenesis and treatment for these diseases. Gene editing has had a disruptive impact in the life sciences, having evolved from the first generation of ZFN, the second generation of TALEN to the current CRISPR. ZFN is a combination of Zinc Finger Protein (ZFP) and FokI endonuclease, which allows for the cleavage of a specific DNA sequence. However, there are disadvantages of off-target effect and low affinity, which leads to limited applications. The second generation of TALEN is based on the modification of the cellular genome by the combination of a DNA-recognising TALEN arm and an artificially modified cleavage domain (FokI) of the nucleic acid endonuclease. TALEN has a DNA recognition domain consisting of highly conserved amino acid repeats, which enables it to recognise target DNA sequences. Compared with ZFN, TALEN

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technology has higher specificity and lower cytotoxicity, and suffers from a relatively complex design and construction process. CRISPR/Cas9 technology is by far one of the most popular gene editing techniques. Its precise editing of target genes is achieved through the use of Cas9 proteins and single guide RNA (sgRNA) in the CRISPR system. Single guide RNA is able to direct the Cas9 protein to localise to the specific site of the target gene and produce DNA double-strand breaks. When repairing these breaks, cells can cause loss of function or mutation of genes through different repair mechanisms. CRISPR/Cas9 technology, with the advantages of high precision, simplicity, efficiency and programmability, has become an important tool and driver for interdisciplinary and translational research, and precision medicine research.

2. Gene editing animal models of neurodegenerative diseases

2.1. Alzheimer's disease

With regard to the cause of Alzheimer's disease, it is now generally accepted that there is an abnormal deposition of beta-amyloid (Aβ) in the brain, forming amyloid plaques (senile plaques) and the abnormal aggregation of Tau proteins to form neurofibrillary tangles, both of which ultimately lead to apoptosis of neurons and synaptic dysfunction in the brain [1]. Common animal models include mice, rats, pigs and non-human primates. Mice are the most commonly used animal model due to their short lifespan, relatively low cost, and well-established genetic manipulation techniques. In mouse animal models such as APP/PS1 and Tg2576, the mouse genome is transfected with human Alzheimer's disease-related genes to mimic the pathological features of Alzheimer's disease.

The APP/PS1 model is a double transgenic mouse model that mimics the pathological features of AD patients in terms of amyloid plaque formation and cognitive decline through the expression of two genes, Mo/HuAPP695swe and PS1-dE9. As the mice grow, abnormal amyloid plaques appear in the brain and cognitive deficits are observed, as evidenced by cognitive and memory decline [2], as well as alterations in neuronal degeneration and synaptic damage, including reduced synaptic density and neuronal loss, are observed in the mouse brain.

Tg2576 mice carry a mutated form of the human APP gene (isoform 695), which contains the Swedish-type mutation (KM670/671NL), a mutation that leads to elevated levels of β-amyloid (Aβ) and, ultimately, the formation of amyloid plaques. This makes the Tg2576 model suitable for studying the role of β in the pathogenesis of AD.

2.2. Huntington's disease

Huntington's chorea is a rare inherited neurodegenerative disease. Patients have a mutation in the Huntingtin (Htt) gene on the short arm of chromosome 4, 4p16.3, which leads to the amplification of the CAG trinucleotide repeat and the production of the mutant Htt protein. The mutant protein is expressed in the brain and leads to neurodegeneration through different molecular mechanisms. It not only contributes to an increase in abnormal function, but also leads to a loss of normal function. Mutated proteins can affect mitochondrial function, intracellular protein transport, autophagy function, etc., which ultimately leads to neuronal cell dysfunction and death. Huntington's disease is an autosomal dominant disorder, with a 50 per cent chance of being inherited by the child if either parent has the disease.

The common mouse genetic model has limitations in mimicking the neuropathology and symptoms of HD in HD, such as the lack of typical nerve cell death characteristics. In response, scientists have carried out research on large animal models. Using CAS9 technology, a team led by Prof Li Xiaojiang of Jinan University and Prof Li Shihua of the Chinese Academy of Sciences, Emory University, USA, inserted everyone's mutated Huntington genes into the endogenous Huntington protein genes of pigs. The results showed that these pigs showed no obvious symptoms until 4 months of age, but gradually developed neurodegeneration and motor deficits similar to those of HD patients as they aged. Pathological analyses also showed that selective neuropathological changes in brain regions of the knock-in pigs were also similar to those in humans. The success of this study not only recapitulates the

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selective neurodegeneration seen in the brains of HD patients, but also provides an important animal model for gene therapy and drug screening in HD and demonstrates the feasibility and importance of gene editing technology for animal models.

2.3. Parkinson's disease

Parkinson's disease is a degenerative disease of the central nervous system that commonly affects middle-aged and elderly patients. The disease is characterised by degenerative defects in dopaminergic (DA) neurons of the substantia nigra striata and Lewy body morphology. The most striking physiological change in the disease is the degenerative death of dopamine (DA) neurons in the substantia nigra of the midbrain. The exact etiology of nigrostriatal degeneration is unknown but is influenced by a combination of environmental influences and genetic factors. Existing Parkinson's treatments fail to treat the root cause of the disease, mainly by replenishing dopamine in the brain or increasing the sensitivity of dopamine receptors, but all of these treatments have side effects, for example, dyskinesia, insomnia and hallucinations. Therefore, the search for new therapies to slow down or stop the progression of the disease has begun and gene editing technology is one of them [3]. Common transgenic animal models for the treatment of Parkinson's disease include Parkin knockout model, α-synuclein overexpression model and LRRK2 gene mutation model.

There are two strategies for the use of gene editing therapies, the first being dopaminergic neuron protection. Since the main pathological feature of Parkinson's disease is the progressive degeneration of dopaminergic neurons in the brain. Therefore, protecting or restoring the function of dopaminergic neurons is the key to treating Parkinson's disease. Therefore, α-synuclein is a therapeutic approach for Parkinson's disease. α-synuclein is able to protect DA neurons from damage by destroying abnormal intracellular α-synuclein filaments, so we can consider α-synuclein vaccine as an effective therapeutic option [4]. Another approach is gene editing therapy to modulate genes related to neuronal survival, proliferation and differentiation to enhance the resistance of dopaminergic neurons. Alternatively, genes can be deleted through gene editing techniques. Scientists have already investigated the therapeutic effects of A53T-SNCA deletion in an in vitro and in vivo viral A53T-SNCA overexpressing rat model of Parkinson's disease using CRISPR-Cas9 tools. When the A53T-SNCA gene was deleted, overexpression of α-synuclein, motor symptoms, dopaminergic neurodegeneration, and reactive microglial cell hyperplasia were significantly reduced, demonstrating the feasibility of gene editing technology in PD treatment [4].

In the face of the treatment of PD, the vectors transporting the editing tools are usually borne by viruses, the reason for this being that viral vector, with their high efficiency of transduction and long term expression, are widely used in therapeutic research in Parkinson's disease. The treatment is similarly divided into two methods: in vitro treatment, in which stem cells are isolated from the human body and genetically edited in vitro before being re-transplanted back into the patient; and in vivo treatment, in which dopaminergic neurons are edited in situ by injecting the gene editing tools directly into the patient's brain. This method requires precise control of the injection location and dosage to avoid damage to normal tissues.

Animal models are not directly involved in the treatment of Parkinson's disease and scientists use animal models to target Parkinson's disease and to understand the pathogenesis, pathology and changes in Parkinson's disease. Neurotoxin models are divided into 6-OHDA model and MPTP model. The former injects 6-OHDA into specific brain regions of animals to simulate dopaminergic neuronal damage and is mainly used for cellular and molecular level studies of PD and evaluation of therapeutic effects. The latter can easily cross the blood-brain barrier and act on specific areas of the brain to cause motor symptoms. This model is characterised by the fact that it best matches the symptoms of PD patients, and that synaptic nucleoprotein deposition can be observed.

Transgenic models use gene editing techniques to mimic the pathophysiological process of Parkinson's disease by overexpressing or knocking out genes associated with Parkinson's disease (e.g., α-synuclein, Parkin, PINK1, etc.) in animals. These models provide an important platform for studying the pathological changes and pathogenesis of PD. The combined model is a combination of neurotoxin

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and transgenic technology to construct a more complex animal model of Parkinson's disease to simulate the pathophysiological features of PD. more comprehensively.

2.4. Amyotrophic lateral sclerosis (ALS)

ALS is a neurodegenerative disease caused by damage to motor neurons, leading to progressive symptoms such as muscle weakness, myasthenia, dysphagia, choking on water and slurred speech. It is mainly divided into sporadic amyotrophic lateral sclerosis (about 95% of cases) and familial amyotrophic lateral sclerosis (about 5-10% of cases), the latter of which is related to heredity and gene mutation. The first symptom is usually weakness and atrophy of the hand muscles, which usually starts from one side and later spreads to the opposite side.

To study the pathogenesis and treatment of ALS, scientists often choose transgenic animal models, most typically the SOD1G93A mouse. This mouse has alanine replaced by glycine at position 93, and this mouse model carries the G93A mutant of the human SOD1 gene, which causes motor neuron degeneration similar to the pathological phenotype of patients with familial ALS. Moreover, scientists have found in mice that if the copper chaperone activity of SOD1 is eliminated, inactivating the SOD1 enzyme, there is no change in the onset or progression of the disease in the SOD1 transgenic model mice, and that the underlying cause of the mutation in ALS is the acquisition of a toxic function, while the mutation in SOD1 is a dominantly inherited mutation in ALS is the underlying cause of the mutation in ALS is the acquisition of a toxic function [5]. Currently, the SOD1G93A mouse model is widely used in preclinical studies of ALS. Knockdown of the mutant SOD1 gene in the SOD1G93A mouse model by the CRISPR/Cas9 system significantly prolonged the survival time and improved the locomotor ability of the mice. Suppressing the expression of mutant SOD1 gene or activating the expression of protective genes by CRISPR/Cas9 system may also be an effective way to treat acromegaly.

3. Cancer gene edited animal models

3.1. Lung cancer

Lung cancer is a malignant tumour that occurs in the lungs or bronchial tubes and is caused by the uncontrolled growth of tissue cells. Lung cancer cells can easily metastasise to adjacent tissues or other parts of the body and the most common symptoms triggered are coughing up blood, weight loss, shortness of breath and chest pain.

The two main types of gene-edited animal models commonly used in lung cancer research include the following: Transgenic lung cancer mouse models: genetic engineering techniques are applied to make mice contain cloned DNA sequences integrated into the genome, thus enabling the production of transgenic mice that pass on genetic alterations to their offspring. For example, a mouse lung adenocarcinoma model was established by using recombinant adenoviral vectors expressing Cre recombinase to induce K-ras G12D expression in mouse lungs, which in turn triggers tumours in lung tissues. The advantage is that one allele of K-ras is replaced by the oncogenic K- rasG12D allele and is expressed only upon somatic recombination with the wild-type allele, thus avoiding early expression of the mutant gene [5]. This model is characterized by its ability to mimic the sporadic features of human lung cancer, which helps to study the development of lung cancer at an early stage. However, its limitations are that the modelling time is difficult to manage, the cost is high, and the quantity is difficult to meet the experimental demand.

Lung cancer transplantation models: Lung cancer transplantation tumour models are mainly divided into homologous animal transplantation tumour models, human tumour cell line xenograft models (CDX) and patient-derived tumour xenograft models (PDX). Homologous animal transplantation tumour models are constructed by inoculating tumour cell lines/lines from homologous background sources into immunocompetent animals. For example, LLC (Lewis mouse lung cancer cells) is inoculated into C57BL/6 mice, which maximally mimics the real-life situation of the tumour microenvironment but may not fully represent the complexity of human tumours in a clinical situation model is a tumour transplantation model constructed by inoculation of a human-derived tumour cell line into an

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immunodeficient mouse. The main inoculation sites are subcutaneous inoculation and in situ inoculation. The subcutaneous inoculation model is time-consuming, inexpensive, and simple to operate, but metastasis rarely occurs; the in-situ inoculation model is more similar to the reality of the microenvironment of tumour growth, but the operation is more complicated and prone to metastasis model: the model is constructed by inoculating the lung cancer of the patient's origin into the immune-deficient mice. The model retains the cellular and histopathological structure of the primary tumour, and the genome and gene expression profile are largely preserved. The predictability of clinical efficacy reaches more than 80%, and it is currently recognized as the oncology research model with the highest accuracy. However, the PDX model is particularly valuable due to its high construction cost, the fact that cancerous tissues or cells from patients are not easily available, and the long time and low rate of tumour formation.

3.2. Breast cancer

Breast cancer is a common malignant tumour in women. Signs of breast cancer include breast lumps, changes in the shape of the breasts, dimpling of the skin, nipple discharge, or red, scaly patches on the skin, among other symptoms.

Scientists utilize mouse models in the study of Breast cancer, which are divided into transgenic mice and knockout mice. Transgenic mice mimic breast cancer by integrating breast cancer-related genes or their regulatory elements into the mouse genome so that the mice express these genes under specific conditions. For example, the MMTV-PyMT transgenic mouse model, which is driven by the MMTV virus to highly express the PyMT oncogene in the mammary gland, leading to malignant proliferation of mammary epithelial cells, which in turn leads to breast cancer. Knockout mice are used to knock out genes related to breast cancer suppression in mice using CRISPR/Cas9 and other technologies, and observe whether mice develop breast cancer and tumour characteristics.

The construction of breast cancer gene editing animal models requires the design of RNA guide sequences and Cas9 nuclease expression vectors for the CRISPR/Cas9 system according to the target genes, and in the next book, the constructed gene editing vectors will be microinjected into the fertilised eggs, so as to make the exogenous genes integrated into the genome of the fertilised eggs. Next, the gene-edited fertilised eggs will be transplanted into surrogate female mice to develop into mice carrying the target gene modification. Finally, the mice carrying the target gene modification were identified by PCR and sequencing, and subsequent breeding and experiments were carried out. By mimicking ILC with Cre recombinase via CRISPR-Cas9, scientists have succeeded in disrupting specific genes in Pten, providing a new opportunity to study the modelling of invasive lobular breast cancer in mice [6].

4. Metabolic disease gene edited animal models

4.1. Obesity

Obesity is a complex metabolic disease involving the interaction of multiple genes and environmental factors. In gene-edited animal models of obesity, researchers can mimic the pathophysiological process of obesity by knocking out or knocking in obesity-related genes, such as leptin (leptin), leptin receptor (leptin receptor), and melanocortin receptor (MC4R). These models help to reveal the pathogenesis of obesity and provide a platform for the development of new therapeutic strategies.

4.2. Diabetes

Diabetes mellitus is a metabolic disease caused by inadequate secretion or defective action of insulin. Gene-edited animal models play an equally important role in diabetes research. For example, NOD mice and BB rats are commonly used in the treatment of type I diabetes. The pathogenesis of IDDM in NOD mice begins with infiltration of the perivascular ducts of the pancreatic islets and the peri-islet area (peri-isletitis) at 3-4 weeks of age. Following this, insulin-producing islet β-cells are slowly, progressively, and selectively destroyed by T-cell-mediated destruction [7]. Animal models of type 1 diabetes can be constructed by knocking out the insulin gene or the insulin receptor gene. In contrast, by knocking out

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genes related to pancreatic β-cell function, such as glucokinase (GCK) or glucagon-like peptide-1 receptor (GLP-1R), it is possible to mimic the pathophysiological process of type 2 diabetes. The BB rat is an ideal animal model for type 1 diabetes because it mimics the natural onset, progression and regression of type 1 diabetes in humans without the involvement or interference of exogenous factors, while the BB rat is a spontaneous hereditary animal model of type C diabetes that has been screened from Wistar rats. These models help to gain insight into the pathogenesis of diabetes and to evaluate the effectiveness of new treatments and drugs.

4.3. Non-alcoholic fatty liver disease

NAFLD is a liver disease that is closely associated with metabolic diseases such as obesity and diabetes. In gene-edited animal models of NAFLD, researchers can simulate the onset and development of the disease in a variety of ways. For example, the onset and development of hepatic steatosis can be observed through an obese mouse model induced by a high-fat diet. Also, scientists have shown that Ldlr --/- mice are also a useful model for studying NAFLD, including liver inflammation and fibrosis, if fed a high cholesterol diet. In addition, the pathogenesis of NAFLD can be further explored by knocking out genes related to fatty acid metabolism, oxidative stress or inflammatory response, such as fatty acid transporter protein (CD36), fatty acid synthase (FASN) or tumour necrosis factor (TNF-α). These models not only help to reveal the pathophysiological process of NAFLD, but also provide an important research platform for the development of new treatments and drugs [8].

5. Conclusion

In summary, gene-edited animal models, as an important tool for modern biomedical research, are gradually playing an irreplaceable role in human disease research. With the continuous development and improvement of gene editing technology, the application scope of gene editing animal models will continue to expand, providing more accurate and effective solutions for the prevention and treatment of human diseases. In the future, gene-edited animal models will play an even more important role in the field of life science and medicine, leading a revolution in the field of biomedicine.

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