

Therapy for neurodegenerative Diseases and expectations

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Abstract. The current development of genetic engineering has been able to intervene in the treatment of many disease areas, especially immune diseases have shown the need for gene editing. The importance of target correspondence is evident at all stages of research. Neurodegenerative diseases have always been a struggling area of medicine, but the current mainstream gene-based immunotherapy has given people the possibility of curing or improving neurological diseases. In the investigation and deepening understanding, at least one immunotherapy has practical application, but the other has not shown the achievements in the direction of neurobiology. Even though immunotherapy is indelible, especially in cancer tumors, the more possibilities of these therapies and the progress of current development are worthy of attention and repeated research. CRISPR therapy is not only able to effectively insert and modify or knock out targeted gene segments in treatment; it is also used to discover the pathogenesis of certain diseases, the pathogenesis suspected by scientists, such as whether the expression level of a certain protein That can be controlled by increasing or decreasing gene expression, both achievable through the implementation of CRISPR therapies. In this review, we summarizes CAR-T and CRISPR therapies in Alzheimer's Disease.

Keywords: Biological Science, Immunity, Gene Editing, Alzheimer's Disease.

1. Introduction

The World Health Organization (WHO) survey shows that the global prevalence of Alzheimer's disease is at a severe level. This disease is closely related to age change, with a trend showing that an average age change of 6.1 years will double the prevalence. In the elderly population over the age of 85, the prevalence of AD can be as high as 20~30%. The number of patients also reveals the urgency of this disease to society. In 2001, the number of AD patients worldwide exceeded 2 million, and it is even expected to exceed 80 million in 2040. AD is the fifth cause of death in the elderly, which not only brings great pain to patients but also heavy mental pressure and medical and care beads to families and societies. The data shows that the cost of AD full-time in 2020 is estimated to be \$604 billion. AD has become a major problem affecting global public health and coastal sustainable development.

There are two types of Alzheimer's disease, familial Alzheimer's disease (FAD) and sporadic Alzheimer's disease (SAD). FAD is an autosomal dominant inheritance, that is, the amyloid premise protein APP gene on chromosome 21, presenilin 1 PS1 on chromosome 14, and presenilin 1 PS2 on chromosome 1. Carrying mutations in APP and PS1 genes will have almost 100% develop into AD, and about 95% of people with mutations in the PS2 gene will develop AD.

In more than 90% of SAD, the gene of apolipoprotein E has a strong correlation. Carriers of the APOE4 gene are at high risk of developing AD. Studies have shown that people who carry one APOE4 allele have a risk of developing AD which is about 3.2 times that of normal people. People who carry two APOE4 alleles are more likely to suffer from AD. The risk of AD is about 8 to 12 times that of normal people.

It is also worth noting the Pathogenesis and pathology of AD. P-amyloid (p-amyloid, Ap) waterfall hypothesis (the amyloid cascade hypothesis) The imbalance between the production and clearance of Ap is the initiation of neuronal degeneration and dementia. The theory of tau protein believes that hyperphosphorylated tau protein affects the stability of neuronal skeleton tubulin, which leads to the formation of neurofibrillary tangles, and then destroys the normal function of neurons and synapses. In recent years, some scholars have also put forward the neurovascular hypothesis, proposing that the

dysfunction of cerebrovascular function leads to neuronal cell dysfunction, and the ability to clear Ap decreases, leading to cognitive impairment. In addition, there are multiple hypotheses such as cell cycle regulatory protein disorders, oxidative stress, inflammatory mechanisms, and mitochondrial dysfunction. Shrinkage and weight loss of the cerebral cortex, deepening and widening of the sulci, atrophy of the temporal lobe - especially the hippocampus. In the coronal section of Alzheimer's disease brain tissue, the bilateral hippocampus was significantly atrophied, the par hippocampal gyrus was narrowed, and the lateral ventricles were enlarged accordingly. The typical histopathological changes are neurotic plaques formed by the deposition of μ amyloid outside the nerve cells and neurofibrillary tangles formed by the accumulation of hyperphosphorylated tau protein in the nerve cells, neuronal loss and glial cells hyperplasia.

2. CAR-T Therapy

This article will briefly discuss CAR-T and mainly discuss the mechanism of CRISPR/Cas9 therapy, as well as the CAR-T therapy is more used for cancer and some inflammatory diseases. Unfortunately, the side effects of CAR-T therapy and the limitations cannot be ruled out for the treatment of neurodegenerative diseases. CAR-T therapies have been approved by the Food and Drug Administration (FDA) [1]. All are approved for the CAR-T therapies have been approved are most used for treatment offing blood cancers, including lymphomas, some forms of leukemia, and most recently, multiple myeloma. Side effects of therapy include cytokine release syndrome and neurotoxicity. CRS is a systemic inflammatory response syndrome (SIRS). When the immune system interacts with pathogens, a large number of white blood cells will be activated and release pro-inflammatory cytokines, and then they may interact continuously, and cytokines and white blood cells are repeatedly activated and released. His limitations are more than that. Regarding its target antigen, CD19 and BCMA are the only antigens approved by the FDA [2]. BCMA is a B cell maturation antigen among them [3]. It belongs to the super ligand family 17 of the tumour necrosis factor TNF (TNF tumour necrosis factor). TNF can kill some virus-infected cells, cause necrosis in tumour cells, or stop tumour cells from receiving nutrients [4]. T-cells, NK cells, and activated macrophages (lymphotoxin LT TNF-) create this tumour necrosis factor. TNF- and TNF- share a receptor despite having just 30% homology. The first cytokine to be employed in tumour biotherapy is this one [5]. It has negative side effects and poor targeting. At the moment, it is solely applied topically.

3. CRISPR/Cas9 Gene-Editing

3.1. Introduction

Clustered Regularly Interspaced short palindromic Repeats Regularly spaced short palindromic repeat (CRISPR) sequence is a repeated sequence in the genome of prokaryotes. It was born because, in cells invaded by viruses, bacteria have evolved the CRISPR/Cas9 system, which is used to excise foreign virus genes that have been integrated into their genetic information [6,7]. Sexual immune system. The so-called acquired immunity refers to only targeting one pathogen. Such acquired immunity can arise through acquired infection or artificial vaccination. The result of specific immunization with a specific antigen can be obtained. The biggest highlight worth mentioning is the special programming enzyme of Cas9 [8]. Efficiency is higher than TALENS and more effective. However, in the treatment of human cancer, there may be a large number of fratricide targets, especially the modification of undesired genes. It is possible to further cause additional risks. Explain in detail how the efficiency is higher than TALENS. TALENS can target longer gene sequences than zinc-finger nuclease-ZFN, but there is no low-cost and published method for the rapid production of large quantities. Another disadvantage that cannot be ignored is that TALENS has certain cytotoxicity and will cause more or less harm. TALENS transcription activation-like (TAL) effector nucleases. Recognition of specific DNA base pairs by means of TAL effectors, a natural protein secreted by plant bacteria. Attaching a nuclease to a TAL effector generates TALENs. Most of the plant

pathogens used are *Xanthomonas monocytophaga*. The proteins naturally secreted by *Xanthomonas* are activator-like effector TAL effector-TALENs. The application method is to design a suitable TALEN to recognize and bind to any characteristic sequence, and attach a DNA double-strand cut at a specific point to a nuclease to construct a TALEN. The purpose of introducing new genetic material into the cell genome can be achieved. CRISPR is a technology that simplifies the modification and customization of induced pluripotent stem cells (iPSCs) [9]. There is a scientific team in 2018 - trying to use CRISPR technology to destroy the HPV gene. CRISPR relies on a guide RNA molecule to guide it to the target DNA and then edits the DNA to disrupt the gene or insert the desired sequence. They are all ready-made, and the total cost is only 30 US dollars, while 96 TALENs a day are 75 US dollars each, and zinc fingers can only be ordered for more than 5,000 US dollars. CRISPR therapy is not only able to effectively insert and modify or knock out targeted gene segments in treatment; it is also used to discover the pathogenesis of certain diseases, the pathogenesis suspected by scientists, such as whether the expression level of a certain protein can be controlled by increasing or decreasing gene expression, both achievable through the implementation of CRISPR therapies.

3.2. CRISPR/Cas9 and Alzheimer's Disease

CRISPR targeting can greatly benefit the treatment of Alzheimer's disease. Targeting γ -secretase, targeting editing of APOE genotype, targeting pro-inflammatory molecules, especially CD33, targeting glial cell maturation factors, and targeting cysteine leukotrienes (a pro-inflammatory lipid molecule).

Now knockout is to be used in CRISPR genome editing. Genomic DNA is perturbed, making them non-functional. Instead of it damages specific genes. By mediating a frameshift mutation which leads to a stop codon to perform near the 5' end of the gene. All transcription downstream of the stop codon.

Wang et al. (2019) reported that down-regulation of Thioredoxin-interacting protein (Txnip) level in HT22 cells via CRISPR/Cas9 system can effectively attenuate amyloid- β -induced protein cysteine oxidative modification [10]. Endogenous γ -secretase was abolished by presenilin1 (PSEN1) gene knockdown in N2a (mouse glioblastoma cells). discovered that the production of Ab42 and Ab40 can be decreased by exogenous addition of recombinant protein obtained from PSEN1 mutations. At this point, I must bring up PSEN 2, a mutation at N141I that might affect starch metabolism and cause a rise in AB42 or a decrease in AB40 [11]. The theory behind the action of calcium ions on α -amylase is that mutations of PSEN1 and 2 can result in high amounts of calcium ions reducing amylase activity. Amylase's activity is inhibited by the competitive inhibition of calcium ions, which bind to amylase or directly bind to the -SH group on amylase. It can be treated as a single treatment point due to the clear link. PSEN2 has a mutation called AB42 that causes it to rise; if the mutation is removed, the substance returns to normal (the part increased due to the mutation returns to the normal level) [12]. It is unavoidable to run into issues when applying actual nerve cells in HT22 and N2a because the gene points to be removed or the aetiology are even different in these two cases. Another factor is the distinct physiology. What will happen when obstacles are directly applied to the therapy of AD in humans is yet unclear. Only the deletion of particular protein expression awakening gene regions is possible at this time.

Another important area for research into the causes of Alzheimer's disease is the immune system. It is impossible to disregard the part persistent neuritis plays in the development of AD. In actuality, CRISPR targets CD33. The pathogenic manifestation of AD can be successfully reduced by mCD33 decreased by decreasing microglia's phagocytosis and increasing the removal of Ab. According to studies, CRISPR/Cas can eliminate the CD33 gene to accomplish this. An acidic protein called glial maturation factor can be affected by different proteases. This pro-inflammatory factor is a recent discovery, and it is mostly expressed in the reactive glia that surround the amyloid plate. Overexpression of GMF leads to the activation of signaling pathways in p38MAPK, which further induces and triggers oxidative toxicity, leading to neuronal cell death. p38MAPK is a stress-activated protein kinase, which is closely related to the quiescence of apoptosis-activated cell cycle in a variety

of tumors, including cervical cancer, ovarian cancer, lung cancer, and lymphoma [13]. When cells are subjected to extracellular stimuli such as inflammation, cytokines, and chemotherapy drugs, the p38 MAPKs pathway is activated to promote apoptosis and inhibit tumor growth. At present, it is believed that the mechanism of p38 MAPK promoting cell apoptosis is by phosphorylating P53, inducing Bax translocation, enhancing the expression of c-myc, participating in Fas/FasI-mediated apoptosis, enhancing the expression of TNF- α , activating c-jun and c-fos and other pathways to induce apoptosis. CRISPR/Cas also targets the Cys LT1R molecule, and again we are introducing an inflammatory cascade -- a molecular complex called NLRP3 that is significantly activated in the brains of Alzheimer's patients. Among them, NLRP3 is an inflammasome, a highly pro-inflammatory cytokine, and NLRP3 can form a large signaling complex with the adapter protein ASC released from cells. Getting to the point, CysLT1R and CysLT2R will start the inflammatory cascade. The deletion of A β 1-42 can cause the increase of CysLT1R. Deletion of CysLT1R by CRISPR/Cas 9 system can successfully and effectively reduce the pathological expression of amyloid, along with the alleviation of neuroinflammation in APP/PS mice, A β 1-42-induced cognitive and hippocampal synapses in APP/PS mice. There was also a corresponding improvement in tactile damage.

3.3. CRISPR/Cas Delivery System in Alzheimer's

Viral delivery methods are the most widely used to deliver CRISPR/Cas release, such as AAV, AdV, and LV [1].

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It is important to keep in mind that adeno-associated virus can induce significant liver toxicity at large doses, thus even though the genetic makeup of AAV's single-stranded DNA will restrict the range of treatment options, it has an inherent advantage in terms of dose reduction. Two open reading frames (ORFs) encoding regulatory proteins and coat antigens are found in the single-stranded DNA genome of AAVs. Inverted terminal repeat (ITR) sequences round the genome. Basically, two ORFs are removed and replaced in AAV vectors with expression boxes for therapeutic proteins or RNAs [15].

4. Conclusions

CAR-T is more expected in cancer treatment, but their current mechanism is not very practical in neurobiology. CRISPR/Cas 9 therapy has greatly helped to explore the etiology and pathogenesis of Alzheimer's disease. At the same time, it slows down and eliminates part of the pathological expression of Alzheimer's disease by targeting different therapeutic genes and awakening gene editing. It is worth noting that AAV, one of the delivery methods of gene therapy in the human body, makes people have to look forward to the performance of retroviruses in the future. The inherent characteristics of retroviruses make them even possible to become the best delivery system for gene

immunotherapy. However, the specific approach and the corresponding side effects still need huge experimental data to explore and support.

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