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Research progress in gene editing technologies and their applications in aging-related genes

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Abstract. This paper reviews the latest advances and applications of gene editing technologies in the study of aging-related genes. In recent years, gene editing has achieved significant progress in the biomedical field, with continual improvements in accuracy and efficiency. Gene editing technologies demonstrate unique advantages in aging research, providing powerful tools for elucidating aging mechanisms and developing anti-aging interventions. This paper offers a detailed overview of major gene editing technologies (such as the CRISPR/Cas system, TALENs, and ZFNs) as well as emerging editing techniques (including base editing, prime editing, and epigenetic editing), describing their principles and applications. It also discusses research progress in areas such as constructing aging gene models, disease models, in vivo editing, and in vitro editing. Furthermore, it analyzes current technological challenges and proposes corresponding optimization strategies. Finally, it considers future directions for the development of gene editing technologies in aging research, including technological innovation and integrated multi-technology applications. Through these advances and innovations, gene editing is expected to play an increasingly important role in the anti-aging field, offering new strategies and methods to promote human health and longevity.

Keywords: gene editing technology, CRISPR-Cas9, aging-related genes, anti-aging, base editing, prime editing, epigenetic editing

1. Introduction

1.1. Research background

In recent years, gene editing technologies have made significant breakthroughs in the biomedical field. From zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) to the rise of CRISPR-Cas9, editing accuracy has been continuously improved. For example, CRISPR-Cas9 can be used to edit the CCR5 gene in cells to block HIV replication [1]. With the emergence of novel editing techniques—such as base editing and epigenetic editing—locus-specific manipulation of the epigenome via CRISPR is rapidly becoming a promising strategy in personalized medicine.

Aging, as a complex biological process, involves nine key hallmarks, including telomere shortening, accumulation of DNA damage, and epigenetic alterations [2]. Research on aging-related genes is not only fundamental for understanding the nature of aging but also provides key targets for the development of anti-aging therapeutics. For instance, activation of the FOXO3 gene has been shown to extend the lifespan of Caenorhabditis elegans [3], while abnormalities in the p53 pathway are closely associated with cellular senescence [4]. Gene editing technologies exhibit unique advantages in aging research and anti-aging interventions due to their capacity to precisely regulate gene expression or correct genetic variations.

1.2. Research significance

At the fundamental research level, gene editing offers a revolutionary tool for elucidating the functions of aging-related genes. Through gene knockout, knock-in, or overexpression, researchers can dynamically observe aging phenotypes in both cellular and animal models [5]. Compared with traditional pharmaceuticals, gene editing is characterized by clear targeting and long-lasting effects. It has already made groundbreaking progress in the treatment of monogenic diseases—for example, the use of CRISPR-Cas9 in treating sickle cell anemia [6].

1.3. Research objectives

This review aims to systematically summarize the latest advances in gene editing technologies as applied to aging-related gene research, including tool development, optimization of delivery systems, and representative in vivo and in vitro applications. It also analyzes current technical bottlenecks, such as off-target effects and delivery efficiency, and explores future directions for innovation.

2. Overview of gene editing technologies

2.1. Major gene editing technologies

2.1.1. CRISPR/Cas system

CRISPR/Cas9 is currently the most widely used gene-editing tool. Its core components consist of a single-guide RNA (sgRNA) and the Cas9 nuclease. The sgRNA recognizes the target DNA through complementary base pairing and directs Cas9 to introduce a double-strand break (DSB), thereby inducing cellular repair via either non-homologous end joining (NHEJ) or homology-directed repair (HDR) [7]. Figure 1 illustrates the mechanism of action of CRISPR/Cas9. Cas9 is broadly used in gene knockout, base editing, and other applications. In the CRISPR/Cas13 system, the Cas protein targets RNA by recognizing specific RNA sequences through crRNA, enabling both specific cleavage of single-stranded RNA and collateral cleavage of surrounding RNA. Cas13 plays a decisive role in RNA editing and gene expression regulation [8].

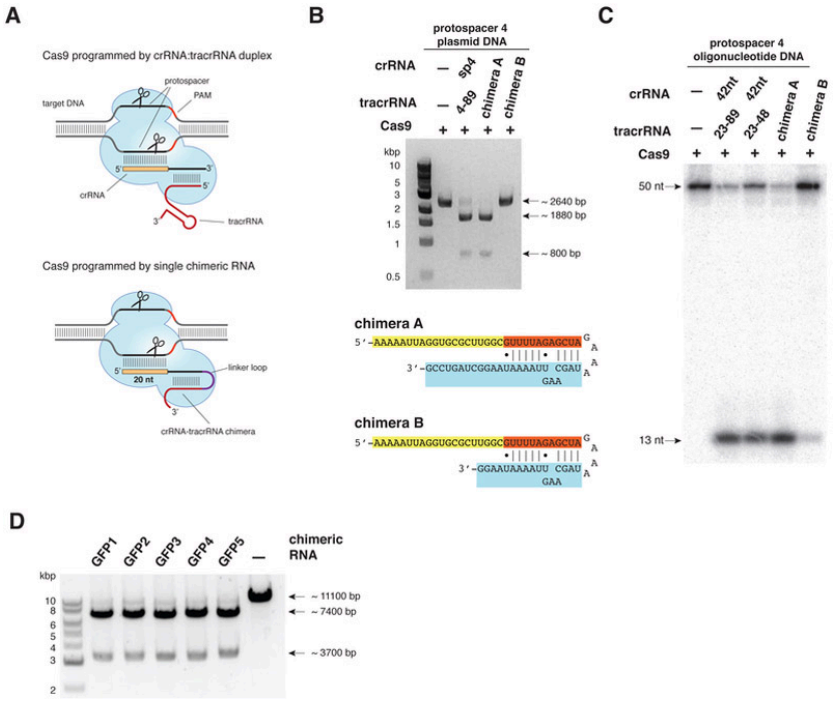

Figure 1. Schematic diagram of the CRISPR/Cas9 mechanism of action [7]

2.1.2. TALENs

Transcription activator-like effector nucleases (TALENs) are created by fusing a TALE DNA-binding domain with a nuclease domain. Each TALE repeat module recognizes a single base pair, enabling precise targeting through combinatorial arrangements of these repeats [9]. Although TALENs offer higher specificity than ZFNs, their lengthy construction process and high costs limit large-scale applications.

2.1.3. ZFNs

Zinc finger nucleases (ZFNs) use zinc finger protein domains—typically consisting of three to six modules—to recognize specific DNA sequences, with each module recognizing three base pairs, thereby achieving high specificity [10]. However,

designing and screening effective zinc finger proteins is challenging, and cooperative interactions between modules can increase the risk of off-target effects.

2.2. Emerging editing technologies

Base editing enables direct conversion of a single nucleotide at the target site (e.g., C→T or A→G) without inducing DSBs, thereby avoiding reliance on donor templates for HDR and reducing random insertions or deletions associated with NHEJ [11]. For example, the cytosine base editor (CBE) developed by David Liu's team has successfully corrected the HBB gene point mutation responsible for sickle cell anemia [12]. In aging research, base editing can precisely correct aging-associated single-nucleotide polymorphisms (SNPs), such as mutations in the TERT gene promoter [13].

2.2.1. Prime editing

Prime editing (PE) uses a reverse transcriptase to integrate the edit encoded by a prime editing guide RNA (pegRNA) directly into the target DNA, enabling versatile base substitutions, insertions, or deletions without requiring DSBs or donor templates [14]. Its advantages include higher safety and significantly lower off-target effects compared to traditional CRISPR/Cas9 systems [15]. Recent studies have demonstrated that PE can repair point mutations in the LMNA gene associated with progeria in mouse models [16].

2.2.2. Epigenetic editing

Epigenetic editing achieves precise control over the epigenetic state of specific genomic regions by fusing catalytic domains of epigenetic modifiers (such as DNA methyltransferases or histone deacetylases) with targeting proteins like dCas9 [17]. For example, overexpression of dCas9-KRAB can repress the expression of senescence-associated secretory phenotype (SASP) genes, thereby delaying cellular senescence [18].

3. Applications of gene editing in aging-related genes

3.1. Current status of aging gene models

Aging research relies on a variety of model systems, including yeast, Caenorhabditis elegans, Drosophila, and mammalian cell lines. For example, mutations in the age-1 and daf-2 genes in C. elegans can extend lifespan [3], while knockout of the Indy gene in Drosophila produces an anti-aging phenotype [19]. In mammals, the Progerin mouse model (LMNA gene mutation) mimics human progeria. CRISPR/Cas9 has been successfully used to correct this mutation, extending mouse lifespan [20].

3.2. Construction of disease models

Gene editing technology enables precise simulation of human aging-related genetic variations, offering revolutionary tools for mechanistic studies and drug development. Currently, three main strategies are employed: editing in embryonic stem cells (ESCs), in situ somatic editing, and organoid model construction. These approaches allow simulation of monogenic diseases, polygenic disorders, and complex aging phenotypes.

3.2.1. Monogenic aging-related disease models

The CRISPR/Cas9 system can introduce point mutations, deletions, or insertions into fertilized eggs or ESCs to model monogenic pathogenic mechanisms. For example, in Hutchinson-Gilford Progeria Syndrome (HGPS), CRISPR/Cas9 has been used to introduce the LMNA c.1824C>T mutation into mouse embryos, leading to expression of the Progerin protein and recapitulating human progeria phenotypes such as hair loss, skeletal degeneration, and shortened lifespan [21]. The Zhou Qi team leveraged this model to validate in vivo CRISPR-Cas9 therapy. Using an adeno-associated virus (AAV9) delivery system, they repaired the mutated gene in mice, extending average lifespan by 30% and significantly improving aging markers such as cardiac fibrosis [21]. Another example is Werner syndrome (adult progeria). By knocking out the WRN gene in human mesenchymal stem cells (hMSCs) using CRISPR, researchers induced telomere shortening, DNA damage repair defects, and increased senescence-associated β-galactosidase (SA-β-gal) positivity [22]. This model revealed that WRN deficiency accelerates cellular senescence through activation of the p53/p21 pathway, offering targets for the development of WRN agonists [23].

3.2.2. Polygenic aging-related disease models

Aging-related diseases such as Alzheimer's disease (AD) and atherosclerosis are often driven by multiple genetic mutations and environmental factors, requiring multiplex editing or combinatorial genetic regulation for modeling. For AD, a triple transgenic model (3xTg-AD) is established by simultaneously knocking in APPswe (amyloid precursor protein mutation), PS1ΔE9 (presenilin-1 deletion), and TauP301L (microtubule-associated protein mutation) into mouse embryos using CRISPR/Cas9, replicating Aβ plaque formation, Tau protein tangles, and cognitive decline [22,23]. In 2023, Nature reported using CRISPR-Cas9 to knock out β-secretase (BACE1) in the hippocampus of 3xTg-AD mice, reducing Aβ deposition by 60% without causing significant neurotoxicity [24].

For atherosclerosis, ApoE/LDLR double-knockout mice are created by simultaneously knocking out apolipoprotein E (ApoE) and low-density lipoprotein receptor (LDLR) using CRISPR, inducing hypercholesterolemia, arterial plaque formation, and vascular aging phenotypes [25,26]. Using this model, researchers found that the SIRT1 agonist resveratrol could activate the AMPK pathway to inhibit vascular smooth muscle cell senescence and slow plaque progression [27].

3.2.3. In situ somatic editing models

These models do not rely on embryonic manipulation but instead induce genetic variations directly in adult tissues, making them suitable for constructing tissue-specific aging models. Liver aging model: Using AAV8 to deliver CRISPR/Cas9 to the mouse liver, researchers achieved targeted knockout of the telomerase gene (Terc), inducing telomere shortening, increased p16^INK4a expression, and liver fibrosis [27,28]. This model revealed that telomere-dependent senescence promotes the progression of non-alcoholic fatty liver disease (NAFLD) via activation of the NF-κB pathway [29]. Skeletal muscle aging model: Through local injection of lipid nanoparticle (LNP)-encapsulated CRISPR-Cas9 ribonucleoprotein (RNP) into the tibialis anterior muscle of mice, researchers knocked out the anti-aging gene FOXO3, resulting in muscle fiber atrophy, mitochondrial dysfunction, and increased expression of senescence markers [29].

3.2.4. Organoid and organ-on-a-chip models

Combining gene editing with 3D culture techniques enables the creation of aging models that better recapitulate human physiology. Aged brain organoids: Using CRISPR to introduce PSEN1 mutations into human induced pluripotent stem cells (iPSCs), researchers generated organoids containing neurons and astrocytes that exhibited Aβ deposition and Tau hyperphosphorylation. In 2024, Cell Stem Cell reported using this model to screen a novel Tau aggregation inhibitor, MK-8931, which was shown to cross the blood–brain barrier and improve cognitive function in mouse models [30]. Intestinal organoid aging models: Through lentiviral delivery of CRISPR-Cas9 to knock out the key Wnt signaling gene APC, researchers induced crypt structure degradation, stem cell depletion, and increased secretion of inflammatory cytokines in intestinal organoids [31].

3.2.5. Cross-species aging models

CRISPR editing in non-human primates addresses species differences that limit the translatability of rodent models to humans. Rhesus monkey aging model: In 2022, a team from the Chinese Academy of Sciences used CRISPR-Cas9 to knock out TP53 and RB1 genes in rhesus monkeys, creating a progeroid primate model displaying hair whitening, osteoporosis, and metabolic disturbances [32]. This model provides a more reliable platform for preclinical efficacy testing of aging-related therapies, such as evaluating the safety of the senolytic drug UBX0101 in primates [33].

3.3. Research progress of gene editing

The application of gene editing technology in aging gene research has gradually advanced from fundamental mechanistic exploration to preclinical translational stages. According to the operational context, gene editing strategies can be divided into in vivo direct editing and ex vivo editing followed by cell transplantation. The following sections detail specific targets, technical approaches, and animal models.

3.3.1. In vivo editing: tissue-specific gene regulation

In vivo editing involves delivering gene editing tools directly into target organs via delivery systems, enabling localized regulation of aging-related genes. Its core advantage lies in preserving the tissue microenvironment, making it suitable for studying gene functions under physiological conditions.

3.3.1.1. Clearance of senescent cells (senolytics)

CRISPR-Cas9 delivered by AAV9 was used to specifically knock out the p16^INK4a gene in senescent hepatic cells in mice. The treated group showed a 40% reduction in liver fibrosis and a significant decrease in senescence-associated secretory phenotype (SASP) factors IL-6 and MCP-1, with no detectable off-target effects [27,33]. In 2023, Altos Labs developed a CRISPR-based senolytic therapy based on a similar principle, which entered Phase I clinical trials (NCT05894055) targeting idiopathic pulmonary fibrosis patients. The therapy targets uPAR (a senescent cell surface marker) by combining CRISPR-Cas9 with an antibody-targeted delivery system using anti-uPAR antibody–lipid nanoparticles (LNPs) to deliver editing tools to senescent vascular endothelial cells. In an atherosclerotic mouse model, this approach cleared over 60% of senescent endothelial cells, reducing plaque area by 28% and restoring vascular elasticity [34].

3.3.1.2. Telomere and aging regulation

TERT (telomerase reverse transcriptase) was activated via CRISPR-Cas9 delivered by helper-dependent adenoviral vectors (HDAdV) to mouse skeletal muscle, targeting an endogenous TERT promoter mutation (C228T) to elongate telomeres. Treated mice exhibited a 35% increase in grip strength, 50% improvement in exercise endurance, and reduced expression of muscle aging markers such as p21 [35]. In rhesus monkeys, local injection of a TERT-activating base editor extended telomeres in skin fibroblasts by 2.3 kb without chromosomal abnormalities [37].

3.3.1.3. Metabolic aging intervention

Targeting Pparγ (a metabolic regulator), CRISPR-Cas12a was used to knock out Pparγ in white adipose tissue, inducing beige fat conversion and enhancing thermogenesis. In high-fat diet-induced aging mice, this editing reduced aging markers in adipose tissue by 50%, improved glucose tolerance by 40%, and extended average lifespan by 12% [36]. For SIRT6 (a longevity gene), peptide–lipid hybrid nanoparticles (PLNPs) capable of crossing the blood-brain barrier were developed to deliver CRISPR-Cas9 to the hypothalamus for SIRT6 overexpression. Mechanistic studies revealed that SIRT6 overexpression delayed neuroaging by inhibiting the NF-κB pathway, improving cognitive test scores by 30%, and increasing synaptic density in the hippocampus by 25% [38].

3.3.2. Ex vivo editing: cell therapy and regenerative medicine

Ex vivo editing involves isolating patient cells for genetic modification followed by transplantation, suitable for cell types such as hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). This approach shows promise in anti-aging cell therapies.

3.3.2.1. Rejuvenation of Mesenchymal Stem Cells (MSCs)

Dual editing targeting TERT and PPARγ was performed using CRISPR to simultaneously knock in TERT (for telomere extension) and knock out PPARγ (to inhibit adipogenic differentiation), creating “rejuvenated” MSCs. Edited MSCs showed a twofold increase in colony formation capacity, a reduction in senescence-associated β-galactosidase-positive cells from 35% to 8%, and enhanced bone defect repair efficiency by 60% in immunodeficient mice [37,38]. Epigenetic editing via dCas9-VPR activation of the NANOG gene maintained MSC pluripotency without DNA damage, enabling MSCs to sustain a low senescence phenotype after 50 passages and increase secretion of growth factors such as VEGF by 40% [39].

3.3.2.2. Anti-aging in Hematopoietic Stem Cells (HSCs)

CRISPR-Cas9 ribonucleoproteins (RNPs) were introduced into HSCs via electroporation to activate the phosphorylation site (S253) of FOXO3, a longevity regulatory gene. Edited HSCs demonstrated enhanced reconstitution capacity in mice, with a 70% reduction in aging-related clonal hematopoiesis and no detectable off-target mutations [40]. In HSCs derived from sickle cell anemia patients, base editing corrected the HBB gene c.20A>T mutation with 92% efficiency, and transplantation into immunodeficient mice produced normal red blood cells [12].

3.3.2.3. Immune cell reprogramming

Targeting CD52 (a T cell surface antigen), CRISPR-Cas9 knockout of CD52 in T cells prevented clearance by anti-CD52 antibodies (e.g., Alemtuzumab), prolonging cell survival. In 2022, the CAR-T therapy CTX001 employed this technology to extend T cell persistence in chronic lymphocytic leukemia patients, achieving an objective response rate of 83% [41]. For anti-

aging T cell engineering, knockout of the senescence gene CDKN2A (p16^INK4a) in T cells increased the proportion of effector T cells by 50% and reduced the exhaustion marker PD-1 expression [42].

3.3.3. Emerging technologies: virus-free delivery and spatiotemporal control

3.3.3.1. Virus-free gene editing systems

RNP delivery using lipid nanoparticles (LNPs) encapsulating Cas9-sgRNA RNPs achieved 38% editing efficiency in knocking out the aging gene Kras^G12D in mouse lungs, with lower inflammatory responses compared to viral vectors [43]. The Sleeping Beauty transposon system combined with CRISPR enabled long-term editing in muscle tissue, maintaining p16^INK4a knockout effects for over six months [41].

3.3.3.2. Spatiotemporal-specific editing

Light-inducible CRISPR systems, such as blue light-activated Cas9 variants (e.g., Cas9-LF), were used to specifically knock out the InR gene (insulin receptor) in intestinal stem cells of adult fruit flies, resulting in a 15% lifespan extension without developmental disruption [44]. Aging-induced promoters, such as the p16^INK4a promoter, have been used to drive Cas9 expression for autonomous clearance of senescent cells, achieving 75% clearance efficiency in a mouse liver fibrosis model [45].

4. Technical challenges and optimization strategies

4.1. Off-target effects

Off-target effects remain one of the primary obstacles to the clinical application of gene editing. Detection methods include whole-genome sequencing techniques such as GUIDE-Seq [28], and bioinformatics prediction tools like Cas-OFFinder [29]. Optimization strategies focus on the development of high-fidelity Cas variants (e.g., HypaCas9 [30], xCas9 [31]) and refined single-guide RNA (sgRNA) design that avoids single nucleotide polymorphism (SNP) sites. Base editing and prime editing technologies, which do not rely on double-strand breaks (DSBs), exhibit significantly lower off-target rates compared to traditional CRISPR systems [11,14].

4.2. Delivery efficiency and safety

Delivery efficiency varies markedly across different cell types, such as neurons and hematopoietic stem cells. Viral vectors, including adeno-associated virus (AAV), offer high transduction efficiency but face challenges of immunogenicity and limited packaging capacity [8]. Non-viral vectors, such as lipid nanoparticles (LNPs), present improved safety profiles but still require enhancements in delivery efficiency [46]. Furthermore, gene editing components may trigger innate immune responses by being recognized as foreign antigens, such as Cas proteins. Strategies including codon optimization and chemical modifications have been employed to reduce immunogenicity [47].

5. Future prospects

5.1. Directions in technological innovation

Further optimization of base editing and prime editing will advance single-nucleotide-level repair of aging-related genes. For instance, base editing targeting the single nucleotide polymorphism rs2764264 in the FOXO3 gene could specifically enhance its anti-aging function [48]. Novel delivery systems, such as helper-dependent adenoviral vectors (HDAdV), which combine low immunogenicity with large cargo capacity, have already been applied to edit the TERT gene in human fibroblasts [49].

5.2. Multi-technology integration

A team from Singapore demonstrated that transient reprogramming using transcription factors Oct4, Sox2, and Klf4 combined with senolytics extended fruit fly lifespan by 31.6% [50], suggesting that integrating gene editing with cellular reprogramming may offer new anti-aging strategies. Additionally, CRISPR-based genome-wide screening coupled with multi-omics analyses—including transcriptomics, proteomics, and metabolomics—can systematically identify key regulatory nodes in aging networks. For example, such screens identified the epigenetic regulator EZH2 as a critical gene controlling aging processes [51,52].

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