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The Applications of Gene Editing Technologies Targeting on *MECP2* Gene to Treat Rett Syndrome

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Abstract. Rett Syndrome (RTT) is a neurodevelopmental disorder, together with side complications including respiratory disorders, seizures, etc. The incidence of RTT is related to age and sex, especially in female children. The characteristic of RTT is that after a period of normal development, children begin to show symptoms of stunted growth and gradually lose their fine hand movements and language ability. Research and evidence indicate RTT is caused by the mutation of the *MECP2* gene and the loss of function of the MECP2 protein. MECP2 protein can collect some related proteins to repress the transcription in order to inhibit RTT. However, RTT cannot be cured with current treatment approaches. Gene editing technologies have been noticed and used in clinical trials. AAV9, CRISPR/Cas9, and 'miRARE' miRNA have been used to treat RTT by targeting the *MECP2* gene, correcting *MECP2* mutation, and restoring the function of MECP2. These gene editing technologies have been approved to improve behavior disorders and prolong survival *in vivo*. This review summarizes the relationship between RTT and MECP2 and gene editing technologies used in curing RTT, aiming to stimulate future studies for clinical applications.

Keywords: Rett Syndrome, MECP2, Gene editing technologies.

1. Introduction

Rett syndrome (RTT) is a severe neurodevelopmental, hereditary X-linked genetic disorder that almost exclusively affects girls and typically appears six months after birth. Over the past two decades, the occurrence of RTT has been roughly estimated at 5-10 instances per 100,000 female individuals [1]. Typical RTT and Atypical RTT are the two main categories of RTT clinical phenotype. The requirement of diagnostic criteria for typical RTT encompasses a regression phase, succeeded by either recovery or stabilization, and is characterized by the complete loss of intentional hand skills, verbal communication, gait disturbances, and the emergence of repetitive hand motions. Atypical RTT refers to conditions that exhibit several, though not all, of the clinical traits associated with classic RTT [1]. The manifestations of the disease also relate to age and the obvious symptoms encompass mid-line hand stereotypies, gastrointestinal dysfunction, autonomic nervous system seizures, autistic features, intermittent breathing abnormalities, scoliosis, autonomic nervous system dysfunction, etc. [2]. Moreover, there are four stages of the progression of RTT: early onset stagnation, rapid developmental regression, pseudostationary period, and late motor deterioration. Due to the decline in language skills, motor functions, and social

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interaction caused by RTT, as well as the lack of specific treatment, the mechanisms of RTT and gene editing treatment require further understanding.

The generation of RTT is mainly associated with spontaneous mutations on the *methyl-CpG binding protein 2* (*MECP2*) gene. MECP2 may be depressed by these mutations and loses normal functions. *MECP2* gene is located on the X chromosome and encodes MECP2. MECP2 mutations have been found in about 95–97% of typical RTT cases and 85% of atypical RTT [1]. MECP2 is significant for inhibiting RTT because it can bind to methylated DNA region and regulates the transcription of thousands of genes. Nowadays, multiple applications and clinical trials have been used to treat Rett Syndrome by targeting MECP2 with gene-editing technologies. For example, AAV9, CRISPR/Cas9, and microRNA are essential and valuable treatments for RTT. The usage of these technologies has remarkable effects in clinical treatment, promising development in the future.

This review summarizes the RTT inhibition function of MECP2 and various of gene technology applications which are used to treat the RTT by targeting MECP2.

2. *MECP2* gene and protein

2.1. MECP2 gene

The regulation function of *MECP2* gene is vital for inhibiting Rett syndrome. MECP2 locates on the Xq28 chromosome band, which is near the interleukin-1- receptor-associated kinase gene and the red opsin gene [3]. This gene can encode and regulate the production of the MECP2 protein.

2.2. Function of MECP2 protein

The expression level of MECP2 protein is closely connected with X-linked developmental disorders. The lack of MECP2 is widely regarded as the main reason of causing the RTT and the high level of MECP2 likely leads to the MECP2 Duplication Syndrome (MDS). MECP2 protein adopts a wedge-shaped structure to bind the non-symmetry methyl binding domain. Biding to CG methylation or non-CG methylation area are both essential MECP2 function (Figure 1). After binding to the methylation domain, MECP2 can collect some other proteins in order to regulate the transcription of following gene like depress their expression. On the one hand, MECP2 protein is able to bind to the CpG dinucleotides of genome and has the ability of mediate repression by interacting with histone deacetylation and corepressor sin3A [3]. On the other hand, MECP2 can also be a transcription activator by binding to the genome cooperating with CREB1. In addition, MECP2 can recognize and bind to CA repeats and regulate the expression of many genes related to neuronal function by modifying the CA region [3]. MECP2 can bind to not only DNA but also RNA.

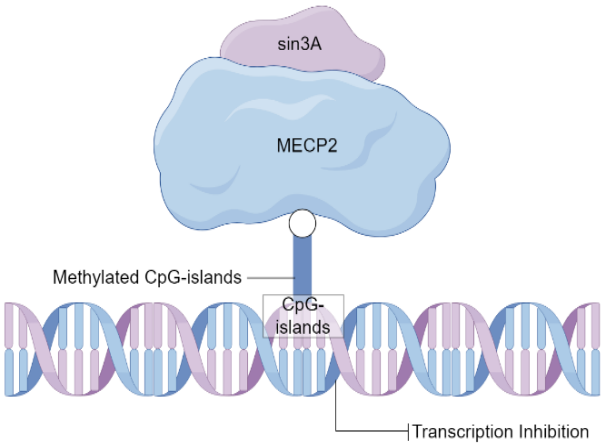

Figure 1. Function of MECP2. MECP2 protein binds to the CpG-island of genome and represses mediate by interacting with histone deacetylation and corepressor sin3A. Created by Figuredraw.com

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2.3. Mutation of MECP2

MECP2 is an epigenetic signals reader, and almost 95%RTT is caused by *MECP2* mutation [3]. *MECP2* mutations cause a variety of phenotypes and disrupt a variety of cell signaling pathways. Genetic mutations in the *MECP2* gene region may change the corresponding protein's ability of biding to the DNA in chromatin. Missense, nonsense, frameshift, and splice site deletions can all make MECP2 lose its function and induce RTT [4]. In RTT, most codon mutations are C to T [4]. Moreover, those mutations affect the MBD of the protein which affects the stability and affinity of its DNA binding.

3. Gene editing technologies used to target *MECP2* gene

Since RTT is a neurodevelopmental destruction disorder and mainly relates to the MECP2, researchers focus on researching some gene editing technologies as treatment approaches. Additionally, studies found that the treatments without targeting MECP2 can only affect on part of RTT. Currently, multiple technologies have been used in treating RTT, including AAV9, CRISPR/Cas9 and microRNA (Table 1).

3.1. AAV9

AAV9 is a viral vector with wide application potential in gene therapy field. When used as the therapeutic vector, AAV9 can efficiently transduce a wide range of cells and tissues in the absence of helper viruses. In recent years, AAV9-MCO has been most used in mouse experiments. After a series of experiments, researchers found this way can actually reduce apnea occurrence and slow movement situation in female RTT mice but is useless for another symptom. However, there are some severe side effects when using this vector. For example, a much higher vector generates off-target effects in mice's bodies and even causes deaths [4]. By analyzing these death mice models, high CHOP levels and TUNEL expression which were detected seriously harm their lives [4]. Off target Mecp2 expression has some probability to led to UPR activation and subsequent apoptosis [4]. Thus, AAV9-MCO can only relieve part of RTT specific to breathing patterns and locomotor deficits but may bring some other unexpected side effects. This method cannot be directly used in human clinical treatment and requires some other auxiliary work to eliminate the adverse events. Besides, scAAV9 is another type of AAV9. By shortening the human MECP2 coding sequence through the modification of the Mecp2 promoter (P546) and incorporating a condensed synthetic poly A terminator, a novel model is constructed to mitigate potential risks [5]. By delivering in the brain, the scAAV9.P546.MECP2 can significantly correct behavior disorders and prolong the life of the mice in experiments in which the *MECP2* gene was knocked out. ICV is the way to deliver scAAV9.P546.MECP2 into the mice brain, some researchers have indicated that it does not affect the movement and survival of WT mice and causes MECP2 over-expression [5]. Thus, scAAV9.P546.MECP2 may be a safe and effective treatment for RTT. This treatment is trying on the clinical stage (NCT05898620), after continuing development this technology may obtain some significant effects in curing RTT.

3.2. CRISPR/Cas9

The type II CRISPR/Cas9 system is an RNA-guided nuclease. Cas9 nuclease can edit genomic sequences by inducting targeted DNA double-strand breaks (DSBs) in mammalian cells. The majority of induced double-strand breaks (DSBs) in DNA are primarily resolved through the non-homologous end-joining (NHEJ) mechanism, which often results in the formation of small deletions or insertions, known as INDELs [6]. Alternatively, the homologous recombination (HR) pathway may be utilized for repair. CRISPR/Cas9 can achieve efficient HR in the human MECP2 locus by using this technology. Moreover, the MECP2 locus can be selectively and specifically modified by CRISPR/Cas9. HR efficiency of 20% to 30% in the MECP2 locus based on the band quantification in former research [6]. Sequencing results from the targeted homozygous clone verified that the accurate alterations were precisely integrated into the *MECP2* gene. There is an efficient CRISPR/Cas9-mediated system which can modify human *MECP2* gene domain and correct the mutation of *MECP2*. In addition to the traditional virus-based CRISPR, there is a novel magnetic nanoparticle-assisted Genome editing

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(MAGE) platform that uses magnetic core-shell nanoparticles (MCNP) to magnetically deliver multiple plasmids encoding the CRISPR-Cas9 system [7]. Magnetic transfection first improves the delivery of polymorphic granulocytes, and then plasmid-containing cells can be purified by magnetic-activated cell sorting (MACS) [7]. This results in increased expression of the Cas9 protein and gRNA, which successfully modifies the target mutant gene. A number of clinical trials have confirmed the feasibility and accuracy of this technology. Thus, CRISPR/Cas9 technology can be a promising therapy for RTT caused by MECP2 gene mutation or low levels of MECP2 protein.

3.3. miRARE

Although all published AAV9/ MECP2 and mini *MECP2* gene therapies have been shown to prolong survival in knockout mice, dose-dependent toxicity occurs when AAV9/ MECP2 is injected into the cerebrospinal fluid of young mice. A tactic to avert the toxicity stemming from excessive gene expression is the incorporation of microRNA target sequences into the 3' untranslated region (UTR) of the viral genetic material [8]. Intrinsic miRNAs have the capacity to bind to complementary sequences within mRNA molecules encoded by the viral genome, thereby diminishing protein synthesis via the mechanism of RNA interference. Furthermore, miRARE can conditionally regulate exogenous MECP2 to reduce the adverse effects caused by excessive miniMECP2 [8]. It not only improves the safety of scAAV9/mini *MECP2* gene therapy, but also does not affect the efficacy of young mice after cerebrospinal fluid injection. TSHA-102 is a AAV9/mini MECP2-miRARE gene therapy, which can be transmitted to the central nervous system between treatment ages, gender and genotype [9]. TSHA-102 improves survival, weight, and breathing during treatment to make it more effective [9]. In summary, miRARE is a widely accepted treatment for *MECP2* mutation and the RTT led by it. It is also a good tool to facilitate other therapies, such as AAV9 technologies.

Table 1. Gene editing technologies used in treating RTT

Technology	Mechanisms	Results	Ref(s)
AAV9-MCO	When used as the therapeutic vector, AAV9 can efficiently transduce a wide range of cells and tissues in the absence of helper viruses.	AAV9-MCO can only relieve part of RTT specific to breathing patterns and locomotor deficits but may bring some other unexpected side effects.	[4]
scAAV9	Truncating Mecp2 promoter (P546) expressing human MECP2 coding sequence and a shorter synthetic Poly A terminator to build the new model and decrease the risk	Significantly correct behavior disorders and prolong the life of the mice in experiments in which the MECP2 gene was knocked out.	[5]
CRISPR/Cas 9	Editing genomic sequences by inducting targeted DNA double-strand breaks (dsbs) in mammalian cells.	Achieve efficient HR in the human *MECP2.* Selectively and specifically modify *MECP2.*	[6,7]
miRARE	Insert microrna targets into the 3′ untranslated region (UTR) of the viral genome.	Prevent gene overexpression-associated toxicity	[8]
TSHA-102	Transmit to the central nervous system between treatment ages, gender and genotype	Improved survival, weight, and breathing during treatment	[9]

4. Conclusion

Researchers have done many related experiments to study its cause and found that the production of RTT is closely associated with the loss of protein function caused by MECP2 gene mutation. Gene editing technologies can selectively target on MECP2 to cure RTT. AAV9 can efficiently transduce

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various types of cells and tissues without helper viruses. scAAV9 is a new model that can truncate the MECP2 promoter and make a short synthetic poly A terminator. CRISPR/Cas9 inducts targeted DNA double helix break to accurately sequences. Using this technology, researchers are able to correct the *MECP2* mutation and help MECP2 protein return to normal function to inhibit RTT. Moreover, inserting microRNA into the untranslated region of the vector virus gene can inhibit the toxicity caused by gene overexpression and can assist the therapeutic effect of AAV9. Besides, miRNA can regulate the exogenous MECP2 and reduce the bad affection caused by miniMECP2. It is a useful way to develop curing safety. TSHA-102 can regulate body weight, breath, and respiration of mice, which can significantly increase their life rate. All these technologies have been used in clinical experiments and have had some remarkable results. Future clinical trials are still needed to continuely prolong the life of the patient, relieve the suffering of the patient, or cure the disease completely. Thus, gene editing technologies are both significant and promising to benefit healthcare in the future.

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