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RESEARCH WATCH

Gene editing: A new step and a new direction toward finding a cure for Duchenne muscular dystrophy (DMD)

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Abstract Duchenne muscular dystrophy (DMD) is a progressive muscle degenerative disease affecting one out of 3500 male births. Patients usually succumb to the disease by age 25. It has been shown that skipping exons of the *DMD* gene that contain disease-causing mutations from the pre-mRNA can result in a shortened, but functional, dystrophin protein that could bring clinical benefits to patients. A recent breakthrough has been reported in *Science* by three groups who demonstrated that genetically deleting exon 23 by gene editing can restore the expression of dystrophin (albeit a shortened version) and improve the muscle function in a mouse model of DMD.

Duchenne muscular dystrophy (DMD) is a relatively common fatal genetic disease that results in muscle degeneration, a loss of mobility, and premature death. There is currently no cure for the disease. The *DMD* gene is located on the X chromosome and is the largest known human gene. *DMD* contains 79 exons and encodes a protein, dystrophin, which is crucial for sarcolemmal integrity. The disease-causing mutations include deletions (present in about 72% of patients) and partial duplications (in about 7% of patients) or point mutations (in about 20% of patients).[1] Some of the mutation-containing exons can be removed, which results in a truncated, but functional gene product with therapeutic effects. For example, removal of exon 51 would be useful to treat 13% of DMD patients. Indeed, it has been

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shown that intramuscular injection of an antisense oligonucleotide induced dystrophin synthesis in four patients with Duchenne muscular dystrophy who had suitable mutations.[1] Although this approach could bring benefits to patients, it is not a permanent solution, because it mitigates genetic defects at the RNA level and requires life-long administration of the antisense oligonucleotides.

Recently, three research articles published in *Science* have described a potentially permanent solution to the problem.[2–4] Using a mouse model of human DMD, the authors have shown that deleting exon 23 of the DMD gene in mice using a gene editing approach could partially restore the dystrophin function in skeletal myofibers and cardiac muscle, leading to significant improvements in muscle function and biochemistry. This was accomplished by using adeno-associated virus serotype 8 or 9 (AAV8 or AAV9) to deliver the clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR associated genes 9 (Cas9)-system to the mice. Two AAV vectors were used, one to deliver the CRISPR-Cas9 enzyme and the other to deliver two guide RNAs specifically targeting the regions flanking exon 23. Since both AAV8 and AAV9 can be used for systemic delivery, both local intramuscular and systemic delivery routes were tested and were found to be able to partially restore the dystrophin expression and muscle function.

This is an excellent example of a case where the CRISPR-Cas9 system can be used, because removal of an exon does not require homologous recombination, and imprecise deletion of a few nucleotides through non-homologous end joining can be tolerated. In fact, more than half of the dystrophin mutations are suitable candidates for custom-designed gene editing to remove mutated exons. The correction of point mutations is also feasible, but remains technically challenging. The efficiency of this approach is expected to be enhanced if a single AAV vector could deliver both the CRISPR-Cas9 and the guide RNAs. Before the application of this approach in patients, the host immune responses to CRISPR-Cas9 need to be tested in large animals. It is already known that the AAV vector and transgene expression can persist for a long time in transduced cells. While continuous expression of the Cas9 system could gradually increase the percentage of cellular DNA to be corrected, it is also associated with the risk of accumulating off-target DNA excisions, and hence, mutations. Improvements are being made that can lead to the development of transient and inducible CRISPR systems of high precision. Taken together, these studies provide a proof-of-principle that gene editing may be useful to treat DMD patients.

Conflict of interest disclosure

XX is a scientific co-founder of Bamboo Therapeutics Inc., which works on DMD gene therapy. The other authors declare no conflicts of interest.

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