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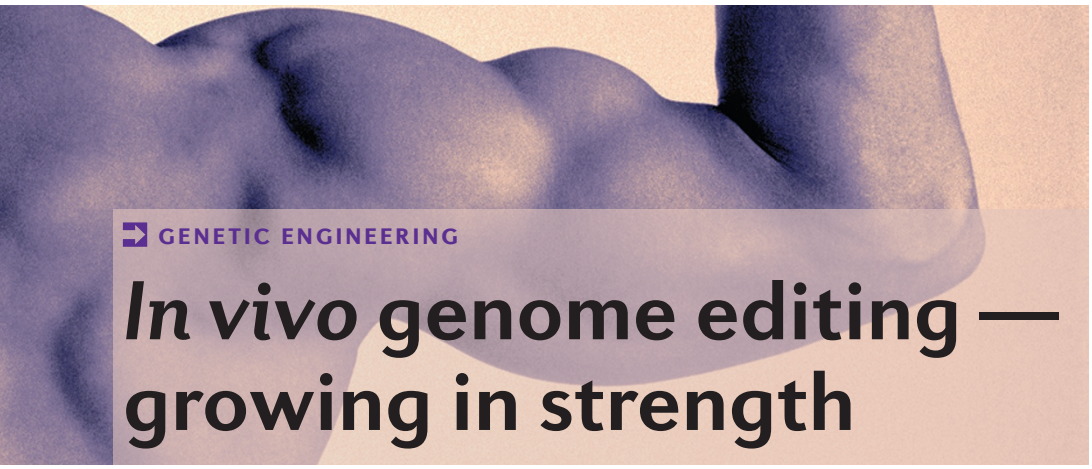

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GENETIC ENGINEERING

In vivo genome editing — growing in strength

> Any genomic modification restricted solely to muscle fibres could risk being lost over time

A trio of independent studies published in *Science* highlights the potential of using the CRISPR–Cas9 system to correct disease-causing mutations after birth, using a mouse model of Duchenne muscular dystrophy (DMD).

DMD is a progressive muscle-wasting disease that results in premature death as a result of mutations in the gene encoding dystrophin. Three research groups — led by Amy Wagers (Harvard University), Charles Gersbach (Duke University) and Eric Olson (University of Texas Southwestern), respectively — had all previously attempted to correct the expression of dystrophin using a variety of approaches. Wagers had shown that transplants of dystrophin-expressing muscle stem cells (satellite cells) into dystrophic muscle can reconstitute the muscle with dystrophin-expressing fibres and satellite cells, but scaling this approach to generate and deliver enough cells to reconstitute every muscle in the body had turned out to be a major challenge. Gersbach had used zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR–Cas9 to restore expression of the dystrophin gene but had focused on correcting the human gene in cultured muscle cells isolated from patients with DMD. "The next major step was to develop a way to correct the gene in muscle tissues *in vivo*," recounts Gersbach. Olson had also previously used CRISPR–Cas9-mediated genome editing to correct the dystrophin gene (*Dmd*) mutation, but in the germ line of mdx mice, which is not a suitable approach to attempt in humans, so the group now turned to postnatal genome editing.

The teams independently developed strategies for the treatment of DMD in the mdx mouse using adeno-associated virus (AAV) to deliver CRISPR–Cas9 to skeletal and cardiac muscle. Cas9-mediated excision of exon 23 of the *Dmd* gene, which harbours the nonsense mutation responsible for the DMD phenotype, restored expression of a truncated version of the dystrophin protein and improved skeletal muscle strength to varying degrees in all three studies after either systemic or local injection. Of note, the amount of restored dystrophin expression observed in mdx mice was within the range expected to provide a therapeutic benefit in humans. Using this exon 'skipping' approach — as opposed to restoring the full-length gene from a DNA repair template — has the advantage of exploiting the non-homologous end-joining pathway, which is active in all cell types, rather than the homology-directed repair pathway, which is less efficient in post-mitotic cells such as skeletal muscle cells. Moreover, correction of the disease-causing mutation need not be precise, as deletions that prevent splicing of mutant exons are sufficient to restore protein expression. Off-target effects of the CRISPR–Cas9 system at other genomic sites nonetheless remain a concern that will require further study.

Interestingly, as assessed by Wagers' team, DMD correction was confirmed not just in myofibres and cardiomyocytes, but also in muscle stem cells. Any genomic modification restricted solely to muscle fibres could risk being lost over time, as new nuclei generated from stem cells without the modified *Dmd* gene are added to fibres in response to damage. "That gene editing can occur in satellite cells *in vivo* means that this approach can create a pool of regenerative cells that now harbour a therapeutically modified *Dmd* gene," explains Wagers, "and participation of these edited cells in the normal process of muscle repair can deliver that edited gene into the fibres so that the modified gene product continues to be expressed."

"The most important work moving forward will be demonstrating safety and assessing immune responses to the delivery vehicle and gene editing system," comments Gersbach. Nonetheless, "these studies represent an important step toward eventual therapeutic application of gene editing for treatment of muscular dystrophy, as well as other monogenic muscle diseases," concludes Olson.

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ORIGINAL ARTICLES Tabebordbar, M. *et al.* *In vivo* gene editing in dystrophic mouse muscle and muscle stem cells. *Science* http://dx.doi.org/10.1126/science.aad5177 (2015) | Nelson, C. E. *et al.* *In vivo* genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. *Science* http://dx.doi.org/10.1126/science.aad5143 (2015) | Long, C. *et al.* Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. *Science* http://dx.doi.org/10.1126/science.aad5725 (2015)