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Nanoparticles for CRISPR–Cas9 delivery

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Abstract

The DNA mutation that causes Duchenne muscular dystrophy in mice can be corrected, with minimal off-target effects, by gold nanoparticles carrying the CRISPR components.

From zinc-finger and transcription activator-like effector nucleases to CRISPR (clustered regularly interspaced short palindromic repeats)–Cas9 (CRISPR associated protein 9), the ability to repair genetic diseases at the DNA level has been a tantalizing goal^{1,2}. The motivation is obvious: a wide range of diseases with genetic aetiology currently have no cure, and the standard of care is based on managing symptoms rather than addressing the root cause. In January 2016, three papers published in *Science* demonstrated how CRISPR–Cas9-mediated correction of a defective dystrophin gene ameliorated the pathology in a mouse model of Duchenne muscular dystrophy (DMD)^{3–5}. The three studies used a technique known as exon skipping, in which paired Cas9 guide RNAs (gRNAs) were designed to target either side of a mutation-harboring exon; as both sites were cleaved simultaneously, the exon was excised from the genome and the ends were joined by an endogenous DNA repair mechanism known as non-homologous end joining. In this process, the mutation is not repaired per se, but rather ‘skipped over’, resulting in a truncated protein that nevertheless retains some biological function. A major limitation of these trials was the use of adeno-associated virus (AAV) as vectors for the in vivo delivery of the CRISPR machinery to cells. Despite AAV-mediated CRISPR delivery resulting in a strong, robust expression of Cas9 and in high levels of editing efficiency, viral vectors have several drawbacks as a clinical-grade therapeutic. From a regulatory perspective, viral therapeutics have historically struggled to obtain approval by the Food and Drug Administration, with only a handful of applications having received approval so far. By comparison, the number of approved protein therapeutics, already in the hundreds, is exponentially growing. The development of better tools to deliver CRISPR proteins directly would thus avoid the need for viral vectors. Reporting in *Nature Biomedical Engineering*, Niren Murthy, Irina Conboy and colleagues now show that functionalized gold nanoparticles can deliver Cas9 and donor DNA both in vitro and in a mouse model of DMD⁶. The approach represents a significant step towards realizing non-viral CRISPR therapeutics.

The nanoparticle formulation of Murthy and co-authors is composed of a gold nanoparticle core surrounded by the CRISPR components necessary for targeted DNA editing — the Cas9 and gRNA ribonucleoprotein (RNP) complex, and the DNA repair template — and

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coated with a cationic polymer that facilitates endosomal escape (Fig. 1a). The authors demonstrate that the nanoparticles are taken up by cells via endocytosis, and that once inside the nucleus the CRISPR components repair the disease-causing mutation directly by replacing the mutated gene with the repair template through an endogenous process known as homology-directed repair (HDR; Fig. 1b). They also show that intramuscular injection of the nanoparticles (together with cardiotoxin, a protein that might indirectly potentiate HDR) in *mdx* dystrophin-deficient adult mice led to the repair of the mutated dystrophin gene. The repair was detectable at the genomic level, with editing efficiency of over 5%, and at the protein level, with dystrophin expression patterns of treated mice resembling those of wild-type mice.

One of the biggest challenges in achieving HDR-mediated genome editing is linked to the fact that endogenous HDR mechanisms are active during cellular division but mostly shut down in terminally differentiated, non-dividing cells. Cardiotoxin was initially included in the treatment protocol because it induces muscle damage, which in turn initiates the proliferation of muscle stem cells and thus should stimulate HDR in these cells. However, whereas its administration would be expected to improve outcomes by promoting editing efficiency through HDR mechanisms, cardiotoxin may not be feasible for translational applications as it causes extensive damage to muscle tissue, and in fact has been used in mice to model the symptoms of muscular dystrophy⁷. As expected, the editing efficiency was reduced when the researchers removed cardiotoxin from the treatment protocol, with the HDR-mediated correction occurring in less than 1% of the transcripts. Interestingly, however, even this low editing efficiency seemed sufficient to partially rescue muscle function in treated mice: in a four-limb hanging test, mice treated with the nanoparticles in the absence of cardiotoxin could support themselves for twice as long as untreated *mdx* mice (yet for significantly less time than wild-type mice).

One of the predicted advantages of non-viral Cas9 RNP delivery is the expectation that off-target editing effects are reduced when compared with AAV-mediated Cas9 delivery. The rationale behind this prospect is purely statistical: delivered proteins will degrade and thus have transient activity, whereas AAV-mediated delivery of the CRISPR machinery leads to constitutive expression of Cas9 and thus increased chances of off-target editing. The results of Murthy and colleagues seem to validate this point. The researchers found off-target genomic damage to be at levels lower than 0.2% (within the error of the sequencing machine used); by contrast, the 2016 studies using AAV-Cas9³⁻⁵ found that off-targeting editing occurred with a frequency of 1.28% (ref.⁴).

There is recent interest in non-viral Cas9 delivery⁸⁻¹⁰ — such as a method for the direct delivery of an engineered Cas9 protein with multiple nuclear localization sequences to improve delivery efficiency¹¹, and the use of lipid nanoparticles for Cas9 RNP delivery^{12,13}. Lipid nanoparticles have also been shown to deliver their payload on systemic injection, and may be engineered or modified to improve their biodistribution on systemic delivery. This possibility is particularly important for the mechanisms of delivery of the CRISPR tools, as it would broaden the range of diseases that may be targeted for gene repair: for instance, whereas intramuscular injection is convenient for the treatment of DMD, treatment of a disease such as cystic fibrosis would require delivery of CRISPR–Cas9 to the lungs.

The study by Murthy and colleagues is the first demonstration of non-viral HDR-mediated CRISPR gene editing in mice. Whereas academic research has demonstrated the promise of CRISPR in treating diseases as wide-ranging as cancer, DMD, cystic fibrosis or even hypercholesterolaemia, the bottleneck limiting the translational potential of CRISPR has consistently been the viral delivery vector. This study broke the barrier of non-viral delivery, and it may facilitate the therapeutic translation of CRISPR treatments in any of these diseases. Recently, early-stage clinical trials have commenced on ex vivo CRISPR therapeutics, in which autologous cells are removed from the patient, edited in the laboratory, and re-introduced into the patient. Non-viral CRISPR delivery may prove to be beneficial for this application as well.

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Fig. 1. Genome editing via nanoparticle-mediated delivery of the CRISPR–Cas9 machinery
a, The nanoparticles are composed of a gold core and a shell of endosomal-disrupting cationic polymer, with the Cas9–gRNA protein complex and the donor template DNA packaged in between. **b**, The Cas9–gRNA ribonucleoprotein generates a cut site at a particular location of the cell’s DNA, and the donor DNA molecule is used as a template to repair the cut site via homology-directed repair (HDR). DSB, double-strand break; gRNA, guide RNA. Figure adapted from: **a**, ref. ⁶, Macmillan Publishers Ltd; **b**, ref. ¹⁴, Macmillan Publishers Ltd.