CRISPR: Established Editor of Human Embryos?

Xiao-Jiang Li1,2,*, Zhuchi Tu1, Weili Yang1, and Shihua Li1,2,*

1Ministry of Education CNS Regeneration Collaborative Joint Laboratory, Guangdong-Hongkong-Macau Institute of CNS Regeneration, Jinan University, Guangzhou, 510632, China
2Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322, USA

Abstract

Off-target effects and mosaicism are major concerns for applying CRISPR-Cas9 to correct genetic mutations. A recent article in Nature by Ma et al. (2017) uses an elegant CRISPR-Cas9 approach that repairs a genetic mutation in human embryos with negligible mosaicism and no off-target effects, bringing this editing tool closer to clinical application.

Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated genes (cas) (CRISPR-Cas9) have now become a simple and versatile RNA-directed system for genome editing in a wide range of different organisms and cell types, including primates and human embryos, to correct debilitating or potentially fatal genetic mutations. However, targeting by CRISPR-Cas9 on approximately 23 base pair matches may generate a number of unwanted or off-target mutations in the genome. Also, CRISPR-Cas9-induced DNA recombination can lead to mosaicism or different types of mutations in disparate cells. Indeed, previous studies by groups in China demonstrated off-target events and mosaic mutations in human embryos that were targeted to specific genes by CRISPR-Cas9 (Liang et al., 2015; Tang et al., 2017). It is widely accepted that off-target effects (off-targets) and mosaicism are the two major hurdles to the application of CRISPR-Cas9 to repair genetic defects in human embryos.

In a recent Nature paper, Shoukhrat Mitalipov and collaborators appear to have identified a promising method to correct a disease-causing mutation in human embryos without mosaicism and off-targets (Ma et al., 2017). They have used CRISPR-Cas9 to target a mutation in a gene called MYBPC3, which accounts for approximately 40% of the myocardial disease hypertrophic cardiomyopathy (HCM). They obtained sperm from a male patient carrying a heterozygous dominant 4-bp GAGT deletion in the MYBPC3 gene and intracellularly injected the sperm into healthy human eggs. Because 50% of sperm alleles from heterozygous patients carry the mutation, half of the developed embryos are expected to carry the mutation as well. The CRISPR-Cas9 components (a single gRNA that selectively targets the mutated DNA sequences, Cas9 protein, and a DNA template for replacement) were injected with the sperm into an egg, resulting in cutting of the mutated DNA and subsequent DNA repair via homology-directed repair (HDR) or non-homologous end-joining (NHEJ). After fertilization, embryos at the 4- to 8-cell stage were collected, and

*Correspondence: xli2@emory.edu (X.-J.L.), sli@emory.edu (S.L.).
each embryonic blastomere was isolated and individually analyzed by DNA sequencing so that the efficiency and rate of genetic mutation repair could be assessed.

In this work (Ma et al., 2017), the authors examined 58 human embryos fertilized with sperm carrying the MYBPC3 mutation and found that 42 (72.4%) contained two normal copies of the MYBPC3 gene. Thorough genome and exome sequencing revealed no evidence of off-target genetic changes and only one embryo carrying a mosaic genotype. These findings alleviate some concerns about CRISPR-Cas9-mediated off-targets that were reported by a group to be widely present in the mouse genome (Schaefer et al., 2017). Thus, the elegant work by Shoukhrat Mitalipov and collaborators appears to overcome the two major problems (off-targets and mosaicism) and represents a significant improvement in efficiency and accuracy in gene targeting over previous efforts. Also, since the authors used healthy human embryos rather than abnormal human embryos as the previous study did, their work brings the technology a step closer to clinical application in correcting inherited diseases.

The reduced mosaicism and off-targets are likely due to a different strategy employed by the authors. First, they used purified recombinant Cas9 protein instead of plasmid DNA or RNA for injection. Second, timing for the addition of CRISPR-Cas9 is also important for reducing mosaicism, as M-phase oocytes simultaneously injected with CRISPR-Cas9 and sperm generated a higher rate (72.4%) of embryos with two copies of the normal MYBPC3 gene than S-phase injected oocytes (66.7%) and uninjected embryos (47.4%). Thus, mosaicism may occur when the genome editing takes place after the DNA has replicated in the egg or after the first cell division. The injected Cas9 protein may also be degraded faster than injected plasmids or mRNA, and a shorter time for Cas9 to cut DNA in M phase can reduce off-targets and mosaic mutations that can occur after zygotic division. In support of this idea, shortening the half-life of Cas9 was found to reduce mosaicism in non-human primate embryos (Tu et al., 2017). Another interesting finding is that newly formed embryos used the maternal copy of the normal DNA as a template to repair the 4-bp deletion mutation, despite the presence of exogenous single-stranded oligodeoxynucleotide (ssODN) templates, and only one of the 42 embryos used the foreign template for repair.

Although the authors present exciting results to show the correction of a genetic mutation without mosaicism and off-targets, their work also opens up some important questions that need to be addressed. First, the targeting efficiency can still be optimized. The yield of embryos consisting of two copies of the normal MYBPC3 gene is 72.4% over the control or untreated (about 50%), which means half of the mutant alleles were not corrected. Thus, the reported approach is mostly useful only when preimplantation genetic diagnosis (PGD) cannot identify normal embryos for transfer. Second, chromatin modification and structures for maternal and paternal DNAs in zygotes are different (Mclay and Clarke, 2003), which may influence CRISPR-Cas9 targeting in eggs and sperm. It remains unclear whether the strategy described by the authors can achieve a similar targeting efficiency to repair maternal DNA mutations. Third, the finding that the endogenous wild-type DNA serves as a template for mutation correction is interesting. Whether this depends on the targeting site or specific DNA sequences or structure remains to be tested. Since genetic variation influences CRISPR-Cas9 targeting (Scott and Zhang, 2017), it is important to validate the current
findings on different genetic mutations and targeting sites. In addition, if the use of an endogenous DNA template to repair DNA is the mechanism for CRISPR-Cas9-mediated DNA repair in human embryos, correction of a homozygous mutation by using the strategy reported in this study would be challenging.

Another important finding by Ma et al. (2017) is the difference in DNA repair between human embryos and stem cells. The authors found that HDR efficiency in patient-derived induced pluripotent stem cells (iPSCs) was significantly lower and primarily achieved through an exogenous DNA template, suggesting that the DNA-repair mechanism or mechanisms in the early embryo may differ from those in the iPSCs and somatic/adult cells. Indeed, the authors found that human MII eggs had a higher rate for HDR, which is different from the theory that HDR is restricted to late S and G2 phases and downregulated at M and early G1 phases (Orthwein et al., 2014). Because there are considerable differences in DNA repairs between human and rodent embryos and higher rates of embryo chromosome instability in primates than any other species (Vanneste et al., 2009), the current work suggests that use of human embryos to validate the process for correction of gene mutation is necessary before clinical application.

It should be pointed out that gene-editing research in human embryos needs to follow appropriate legal and ethical regulations and is not currently supported by the US National Institutes of Health. Non-human primates are the closest species to humans and have been used to model human disease via CRISPR-Cas9-mediated gene editing (Chen et al., 2015). Given these considerations, non-human primate embryos are an important source for scientists to investigate the mechanisms underlying DNA repair in zygotes and to validate potential gene therapy via CRISPR-Cas9 in germline cells.

References


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