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·**Research Highlight**·

TALEN-mediated gene mutations in monkeys

Yinghui Zheng, Xiao-Jiang Li

State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

Corresponding author: Xiao-Jiang Li. E-mail: xjli@genetics.ac.cn

Genetic manipulation by targeting defined genomic loci allows the generation of precise mutations in the genome in animal models that can recapitulate genetic defects in human diseases caused by inherited or *de novo* mutations. However, most of the previous gene-targeting manipulations have been restricted to mice, because their embryonic stem cells are available and can be modulated at defined genomic loci[1]. The recent discovery and application of sequence-specific endonucleases, namely transcription activator-like effector nucleases (TALENs) and RNA-guided nucleases (CRISPR/Cas9), have revolutionized genome manipulation in numerous model organisms[2, 3]. In this issue, Liu *et al.* report the use of TALENs to successfully generate targeted mutations of *MECP2* in cynomolgus monkeys.

The *MECP2* gene encodes a methyl-DNA-binding protein that represses gene transcription and plays an important role in mammalian development. It appears to be particularly important for mature neurons. *MECP2* is located on the long (q) arm of the X-chromosome, and its mutation in humans can lead to Rett syndrome, a severe neurodevelopmental disorder[4, 5] and one of the most common causes of mental retardation in females, with a prevalence of ~1 per 10,000. The *MECP2* locus is X-linked, and the disease-causing alleles are dominant. As a result, *MECP2* mutations have been linked to male lethality. This year, two independent findings[6, 7] in China demonstrated that TALENs are able to create mutations in *MECP2* in monkeys; one by Liu *et al.* from the Institute of Neuroscience, Chinese Academy of Sciences[6].

Like most investigators, Liu *et al.* injected TALEN mRNA into the cytoplasm of one-cell embryos and generated 5 offspring, one of which was positive for mutations in *MECP2*, giving a 20% targeting rate in monkeys. The mutations appear to be mosaic in the tissues. Also, the male monkey with the *MECP2* mutations did not survive after birth. This appears to be consistent with the earlier report on TALEN-mediated mutations in the monkey *MECP2* gene[7].

However, there are differences between these two studies. First, the deletion of *MECP2* represents a typical TALEN-mediated mutation[6], whereas in the mutant monkey reported earlier, the majority were point mutations (single nucleotide exchanges) that were also present in non-targeted DNA regions[7]. The earlier report showed a higher targeting rate; all monkeys examined (two miscarried male fetuses and one live female) had multiple point mutations in the *MECP2* gene[7]. Such mutation differences could be due to the different approaches used. In the study by Liu *et al*., TALEN mRNA was injected into fertilized oocytes[7] whereas in the earlier study TALEN plasmids with RAD51, which assists in the repair of DNA double-strand breaks, were injected into the cytoplasm of fertilized oocytes[8]. Thus, the cytoplasmic injection of TALEN plasmids with RAD51 may yield a much higher mutagenesis rate in the genome. Another difference is that Liu *et al.* reported that monkeys with TALEN mutations can survive to full term (160 days of gestation) with grossly normal brain development, whereas in the earlier report two male fetuses carrying *MECP2* mutations were dead at day 63 of gestation[7]. Although both studies indicated male lethality, whether and when the loss of *MECP2* causes male lethality remain to be investigated, as both studies showed mosaic mutations in aborted monkeys. However, there were also aborted monkeys without the *MECP2* mutations, which could result from TALEN mRNA toxicity or *in vitro* fertilization

and embryo transfer manipulations[6]. The brain tissues with *MECP2* mutations in the study of Liu *et al.* would be valuable for further investigation of the above issue.

The study by Liu *et al.* further verified that TALENs can be used in non-human primates to generate gene-targeted mutations. This finding, combined with a recent report using CRISPR/cas9 to target monkey genes[8], demonstrates the potential to generate non-human primate models of human diseases. Since cytoplasmic injection of TALEN mRNA *versus* the cytoplasmic injection of circular plasmid TALEN DNAs with RAD51 can lead to very different gene targeting and consequences, these differences remain to be understood so as to identify an efficient approach for precise gene targeting in the monkey genome. Apparently, there is much room for improving gene-targeting efficiency and overcoming the mosaic mutation issue found in gene-targeted monkey models.

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