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LETTER

Effective and precise adenine base editing in mouse zygotes

Dear Editor,

Many human genetic diseases are caused by pathogenic single nucleotide mutations. Animal models are often used to study these diseases where the pathogenic point mutations are created and/or corrected through gene editing (e.g., the CRISPR/Cas9 system) (Komor et al., 2017; Liang et al., 2017). CRISPR/Cas9-mediated gene editing depends on DNA double-strand breaks (DSBs), which can be of low efficiency and lead to indels and off-target cleavage (Kim et al., 2016). We and others have shown that base editors (BEs) may represent an attractive alternative for disease mouse model generation (Liang et al., 2017; Kim et al., 2017). Compared to CRISPR/Cas9, cytidine base editors (CBEs) can generate C•G to T•A mutations in mouse zygotes without activating DSB repair pathways (Liang et al., 2017; Kim et al., 2017; Komor et al., 2016). In addition, CBEs showed much lower off-targets than CRISPR/Cas9 (Kim et al., 2017), making the editing process potentially safer and more controllable. Recently, adenine base editors (ABEs) that were developed from the tRNA-specific adenosine deaminase (TADA) of *Escherichia coli* were also reported (Gaudelli et al., 2017). As a RNA-guided programmable adenine deaminase, ABE can catalyze the conversion of A to I. Following DNA replication, base I is replaced by G, resulting in A•T to G•C conversion (Gaudelli et al., 2017; Hu et al., 2018). The development of ABEs has clearly expanded the editing capacity and application of BEs. Here, we tested whether ABEs could effectively generate disease mouse models, and found high efficiency by ABEs in producing edited mouse zygotes and mice with single-nucleotide substitutions.

Unlike CBEs that can generate premature stop codons with C-T conversion (TAG, TAA or TGA), ABEs cannot produce a new stop codon to disrupt gene function via A-G conversion. We therefore targeted mRNA splice sites in order to induce gene dysfunction. Since mammalian mRNA splicing requires a 5′ GU donor and a 3′ AG acceptor at intron-exon junctions, ABEs can block mRNA splicing and hence inactivate gene function by converting splice donors and acceptors to GC and GG. We named this strategy ABE-induced mRNA splicing defect (AI-MAST).

We first used ABE7.10 to target the mouse *Tyr* gene, whose dysfunction results in albinism in mice (Zhang et al., 2016). A gRNA was designed to target the splice donor at exon 3 of the *Tyr* gene, which is also predicted to be an ideal site for ABE. We then injected both ABE7.10 mRNA and the gRNA into mouse zygotes (Fig. S1A). Of the 20 embryos harvested 48 h later, 9 were edited (45.0%) with efficiencies ranging from 11.2% to 24.6% (Fig. S1B–D). In addition, 106 injected zygotes were transplanted into pseudopregnant mothers. Among the 23 pups obtained, 13 (56.5%) showed A-to-G editing with conversion frequencies of 14.6%–48.1% (Figs. S1B and S2), attesting to the feasibility of AI-MAST in generating point mutations in mice.

It should be noted that we did not obtain any white-coated F0 mice, likely due to insufficient A-to-G conversion rate at the splice donor site. However, when the T1–12 F0 mouse was mated with homozygous *Tyr* mutant (c.655G>T, *p.E219X*) C57BL/6J mice (Liang et al., 2017), 2/5 (40.0%) pups were albino (Fig. S3A). Sanger sequencing results indicated that the 2 albino pups were compound heterozygous for both the ABE target site and *Tyr* site (c.655G>T, *p.E219X*) (Fig. S3B), lending support to *Tyr* gene dysfunction as a result of A•T to G•C conversion at the splice donor of exon 3. Furthermore, analysis of RNAs extracted from the skin of these compound heterozygous mice found significant reduction of correctly spliced *Tyr* mRNAs compared with $Tyr^{E219X/+}$ mice (Fig. S3C and S3D). Both $Tyr^{E219X/+}$ and $Tyr^{E219X/E219X}$ mice showed obvious reduction of *Tyr* mRNA, indicating that Tyr^{E219X} mutant RNA is subjected to degradation by nonsense-mediated mRNA decay (NMD). These data demonstrate that AI-MAST is capable of inducing mRNA splicing defects. However, whether phenotypes associated with mRNA splicing defects can be observed in F0 mice remains unknown.

To further explore one-step generation of disease mouse models using ABEs, we designed two gRNAs that targeted the splice sites at exons 61 and 66 of *Dmd* (Fig. 1A). These two sites were chosen because Dunchenne muscular dystrophy (DMD) remains a progressive neuromuscular degenerative disorder with no effective treatment. The largest in the human genome with 79 exons and 2.4 Mb long, the human *DMD* gene has recorded thousands of mutations (2,898 in the UMD-DMD database for DMD patients), including insertions, deletions, duplications and point mutations. At least 158 splice site mutations have been identified

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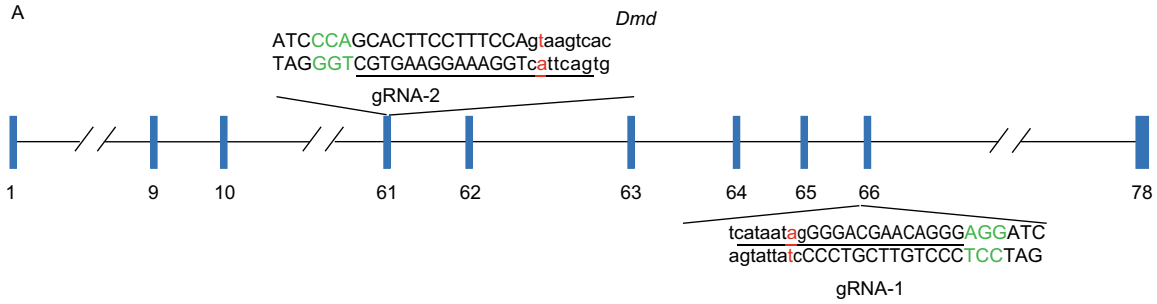

B

gRNA group	Genotyped embryos (#)	Mutant embryos (#)	Transferred embryos (#)	Total pups (#)	Mutant pups (#)	Mutant pups with >95.0% efficiency (#)
1	18	15 (83.3%)	270	67 (24.8%)	47 (70.1%)	14 (20.9%)
2	17	9 (52.9%)	122	26 (21.3%)	11 (42.3%)	1 (3.8%)

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