Dear Editor,

There are hundreds of disease-causing single-gene mutations, mainly caused by single-nucleotide substitutions or point mutations rather than small insertions/deletions (indels), and often there are no cures for these diseases. By introducing the CRISPR/Cas9 system into mouse zygotes, disease-causing mutations could be corrected, leading to the production of healthy adult animals (Long et al., 2014; Wang et al., 2013; Wu et al., 2013; Yang et al., 2013). Several studies have demonstrated that CRISPR/Cas9-mediated gene editing could also introduce precise genetic modifications in early human embryos (Kang et al., 2016; Liang et al., 2015; Tang et al., 2017). However, indels rather than single-nucleotide substitutions are obtained frequently, because most DNA double-strand breaks (DSBs) produced by programmable nucleases are repaired by error-prone non-homologous end-joining (NHEJ) rather than homologous recombination (HR) using a template donor DNA. Recently, base editors, composed of cytidine deaminase, Cas9 nickase (nCas9), and uracil DNA glycosylase inhibitor (UGI), have recently been developed to substitute a C at a target site with T without generating DSBs in plant, yeast, mouse zygotes, and human cells, and shown to be >100-fold more efficient than HR at generating point mutations (Kim et al., 2017a; Kim et al., 2017b; Komor et al., 2016). Several studies have demonstrated that CRISPR/Cas9-mediated gene editing could also introduce precise genetic modifications in early human embryos (Kang et al., 2016; Liang et al., 2015; Tang et al., 2017). However, indels rather than single-nucleotide substitutions are obtained frequently, because most DNA double-strand breaks (DSBs) produced by programmable nucleases are repaired by error-prone non-homologous end-joining (NHEJ) rather than homologous recombination (HR) using a template donor DNA. Recently, base editors, composed of cytidine deaminase, Cas9 nickase (nCas9), and uracil DNA glycosylase inhibitor (UGI), have recently been developed to substitute a C at a target site with T without generating DSBs in plant, yeast, mouse zygotes, and human cells, and shown to be >100-fold more efficient than HR at generating point mutations (Kim et al., 2017a; Kim et al., 2017b; Komor et al., 2016).

Using engineered base editors containing mutated cytidine deaminase domains, such as YE1-BE3, may narrow the width of the editing window (Kim et al., 2017b). Yet, the efficiency and specificity of base editors has not been demonstrated in human embryos. Here, we report that both base editor 3 (BE3) using nCas9 and SaKKH-BE3 using SaKKH-nCas9 can introduce single-nucleotide substitutions efficiently in human tripronuclear (3PN) zygotes.

We first used BE3 (rAPOBEC1-nCas9-UGI) to induce point mutations in human β-globin (HBB), which associated with human diseases β-thalassemia (Fig. 1A and 1B). We expected to introduce a premature stop codon in HBB by G-to-A conversions at the target site. We carried out base editing in human 3PN zygotes by microinjection of BE3 mRNA and sgRNAs. The injected 3PN zygotes were cultured into 4 to 8-cell embryos and used for targeted-deep-sequencing analysis. Targeted point mutations were observed in 8 out of 19 (42%) embryos at the target site in the HBB gene, with mutation frequencies that ranged from 6% to 52% (Figs. 1C, 1D, 1K, and S1A). Targeted deep sequencing showed that 7 out of 8 embryos for HBB base editing contained a nonsense mutation at the target site, generated by a single G-to-A conversion (Figs. 1D and S1A).

To broaden the genome-targeting scope of base editors, we used the recently reported SaKKH-BE3 that relaxes the variant’s PAM requirement to NNNRRT (Fig. 1A). Targeted deep sequencing on the injected embryos revealed that 17 out of 17 (100%) or 6 out of 9 (67%) embryos carried targeted point mutations at the target site in the FANCF or DNMT3B gene, respectively (Fig. 1E–K). Note that we observed very low percentage (<5%) of wild-type (Wt) allele in 5 FANCF mutant embryos (FANCF-E2, E7, E9, E11, and E17) and no Wt allele in 3 FANCF mutant embryos (FANCF-E13, E14, and E15), indicating high base-editing efficiencies in human 3PN embryos using SaKKH-BE3 (Figs. 1G, S1B, and S1C). Targeted deep sequencing showed that a C-to-T conversion was the major mutagenic pattern at all three target sites, with frequencies range from 78.8% to 98.5% (Fig. S2). C-to-A or C-to-G conversions were also observed in 8 out of 19 (42%) embryos at the target site in HBB sequencing showed that 7 out of 8 embryos for HBB mutant embryos (Figs. 1 and S1). We also found C–T conversion on the upstream or downstream of the sgRNA target site in 0 HBB (0%), 10 FANCF (59%), and 3 DNMT3B (50%) mutant embryos (Fig. 1E–K), consistent with previous studies (Kim et al., 2017a; Kim et al., 2017b; Komor et al., 2016).

Using engineered base editors containing mutated cytidine deaminase domains, such as YE1-BE3, may narrow the width of the editing window (Kim et al., 2017b). To avoid the PCR bias, we further examined 4- to 8-cell embryos with FANCF or DNMT3B base editing at the single-cell level. Single blastomeres of 4- to 8-cell embryos were isolated and picked up under the microscope for PCR amplification and Sanger sequencing. We found that 10 out of 10 (100%) or 9 out of 9 (100%) embryos carried targeted point mutations at the target site in the FANCF or DNMT3B gene, respectively (Figs. 1K, S3, and S4). Based on single-cell sequencing reads, 79% or 83% alleles carried targeted point mutations in the FANCF or DNMT3B (Fig. S5). Among these mutant embryos, two FANCF base-editing embryos...
FANCF-E20, E24) and two DNMT3B base-editing embryos (DNMT3B-E11, E14) contained only targeted point mutations (Figs. S3 and S4). C-to-T conversion was the major mutagenic pattern, and C-to-A or C-to-G conversions were also observed in FANCF and DNMT3B mutant embryos (Fig. S5).

Furthermore, compared with CRISPR/Cas9-mediated gene editing (Kang et al., 2016), although 7 out of 10 FANCF mutant embryos contained indels alleles (Fig. S3), the percentage of total DNA alleles with indels was very low (13% for FANCF and 0% for DNMT3B) (Fig. S5D). Further optimizing base editors with inactive Cas9 mutant or Cpf1 mutant may reduce the indels to a lower level.

Finally, to assess base editors off-target effects, we performed whole genome sequencing (WGS) to identify SaKKH-BE3 off-target mutations in the three FANCF mutant embryos (FANCF-E28, E29, and E30) (Fig. S6). Of 1,187 possible off-target sites that differ from the on-target site by up to 5 mismatches, we observed just 1 potential off-target

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**Table:**

| Target gene | Base editors | No. of injected embryos | No. of two-cell stage embryos (%) | Mutant ratio (%) | No. of mutants/total embryos |
|-------------|--------------|-------------------------|----------------------------------|-----------------|------------------------------|
| HBB         | BE3          | 22                      | 19 (86)                          |                 | 8/19 (42)%                   |
| FANCF       | saKKH-BE3    | 18                      | 17 (94)                          |                 | 17/17 (100)%                 |
| DNMT3B      | saKKH-BE3    | 9                       | 9 (100)                          |                 | 6/9 (67)%                    |
| FANCF       | saKKH-BE3    | 10                      | 10 (100)                         |                 | 10/10 (100)%                 |
| DNMT3B      | saKKH-BE3    | 9                       | 9 (100)                          |                 | 9/9 (100)%                   |

*PCR products of whole embryos were used for targeted deep sequencing.*

*PCR products of single blastomere from divided embryos were used for Sanger sequencing.*
site in 1 out of 3 FANCF mutant embryos (Fig. S6). Taken together, these results indicate that BE3 did not induce significant off-target alterations in gene-edited human embryos.

In summary, our results show that microinjection of BE3 or SaKKH-BE3 mRNA resulted in efficient and precise base editing in human 3PN zygotes. These results demonstrate that base editors can be used for correcting genetic defects in human embryos in the future.

FOOTNOTES

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