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Modelling human point mutation diseases in *Xenopus tropicalis* with a modified CRISPR/Cas9 system

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Ever-increasing accumulation of enormous human genetic variants with the advent of high-throughput DNA sequencing challenges sequence interpretation and variant classification, which hampers the advancing of genomic medicine. It is highly desirable to systematically evaluate and study the pathogenic variants in a suitable cost-effective *in vivo* model. The true diploid frog *Xenopus tropicalis* is showing its power in modeling human diseases with high efficiency and penetrance. However, the currently available knock-in method in frogs is still inefficient to achieve precise point mutations, which account for the majority of human genetic diseases. Recently, CRISPR/Cas9-cytidine deaminase fusion has been proven being an effective single nucleotide editing tool. Here we report the efficient conversion of C to T (or G to A) in *X. tropicalis* using the CRISPR/Cas9D10A-AID/APOBEC-UGI base editor. Co-injection of gRNA and the Cas9 mutant complex mRNA into 1-cell stage X. tropicalis embryos caused precise C to T substitution in 13 of 19 targeting sites tested with efficiencies of 25-55%. Targeting the conserved *tyrosinase (tyr)* codon 28 resulted in 20% tyr^{C28Y} genotype and mosaic albino phenotype in G0 frogs, recapitulating the human albinism. Four targeted sites (tyr^{C28Y}, $sftpb^{Q5*}$, $kcnj2^{L382L}$, and *ptf1a/p48*) analyzed all showed successful delivering of the C to T conversion to F1 offspring with 14-67% germ line transmission rates. Our data indicate that the Cas9-cytidine deaminase fusion is an easy and efficient tool for precise base editing in *X. tropicalis,* expanding the utility of this diploid frog for modelling human point mutation diseases.

Biography

Yonglong Chen has his expertise in frog endoderm development. Using Xenopus as a model, he defined the key role of Hhex in specifying the precursors of ventral pancreatic buds during gastrulation. He also found two strategies that can easily expand pancreas mass and islet beta cell population *in vivo*. Recently, his group has established efficient gene knockout and knock-in methods in Xenopus tropicalis, which allows to model human genetic diseases in this diploid frog.

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