The potential of CRISPR base editing for a one-fits-many gene editing therapy

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Background

Gene editing by means of CRISPR base editing (BE) is a novel technique with great potential to cure inborn errors of metabolism due to its (1) permanent nature by direct gDNA editing, (2) versatility in the types of possible edits, and (3) absence of double-strand breaks and the associated risk for chromosomal rearrangements. Previous studies demonstrated high editing rates *in vivo* when delivered to the liver and recently, the first ever documented use of BE in an infant with neonatal onset *CPS1* deficiency was published and reported even in mainstream media. Nevertheless, its widespread use in clinics for correcting pathogenic variants proves difficult due to the large diversity of private mutations and the associated challenges in designing, testing and approving such highly personalized therapies. Therefore, we propose a novel approach of "stabilizing gene editing", where, exemplified on the ornithine transcarbamylase (*OTC*) gene, we identified protein stabilizing variants (PSV) which are able to compensate the negative effect of pathogenic variants. However, none of these PSV are introducible by BE, which has generally been shown to exhibit higher editing rates than other gene editing methods, which makes it more attractive for clinical applications. Therefore in this study, we designed a library of all possible edits in the ORF of the *OTC* gene accomplishable by BE and cloned it into a lentiviral (LV) plasmid allowing expression of a fluorescent-tagged OTC.

Methods & Results

The resulting LV library of >500 substitution edits, each spanning over 1-3 *OTC* codons, was used to transduce a HEK-based cell line followed by FACS-sorting of highly fluorescent clones for identifying stabilizing variants after verifying OTC activity in these clonal populations. The top hits were re-cloned and validated in a second experiment where stabilization was confirmed by Western Blot and OTC activity assays. Furthermore, gene editing by BE was explored to confirm the editability of these stabilizing variants *in vitro*.

Conclusion

In summary, we have identified new variants for a potential stabilizing gene editing therapy for *OTC* deficiency caused by destabilizing mutations using CRISPR BE. If by this method, enzyme function can be restored in an *in vivo* model of *OTC* deficiency, currently ongoing in our lab, it poses great potential for a one-fits-many gene editing therapy circumventing the need for highly personalized gene therapy.