GeNeXpreSS

How Far Have We Progressed in Cutting and Pasting the Genome?

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Redrafting the genetic code in diploid human embryos (Tang et al, 2017)

CRISPR/cas9 was proposed to have the ability to correct disease- causing mutations by gene editing. Previous studies done showed that the efficacy of homology directed repair (HDR) was only 10% when CRISPR/ cas9 was used in triploid human embryos. In this study, the authors have performed cas9-mediated gene editing in diploid human embryos with point mutation in the *G6PD* and *HBB* genes. They have shown that there was HDR in 100% of embryos (two embryos were used) with a mutation in *G6PD*. Out of this, one embryo showed complete correction but the other became a mosaic with 50% cells demonstrating 4bp deletion near the mutation. In this study 50% HDR for *HBB* gene was demonstrated. Only one corrected embryo was free of off-targeting. The authors concluded that the use of CRISPR/Cas9-mediated gene editing in reproductive clinics is not a current option due to both ethical and technical issues like safety and mosaicism. The authors suggested that CRISPR/Cas9 system could be used in human zygotes to study gene function in preimplantation development.

Fine tuning the process of editing human embryos (Ma et al, 2017)

Hypertrophic cardiomyopathy (HCM) is a myocardial disease characterized by left ventricular hypertrophy, with an estimated prevalence of 1:500. It is the most common cause for sudden cardiac death in young adults. Variations in *MYBPC3* account for approximately 40% of all genetic defects causing HCM. This gene is also implicated in other inherited cardiomyopathies, including dilated cardiomyopathy and left ventricular non-compaction. In this study, using CRISPR/ cas9, authors specifically targeted a heterozygous four-base-pair deletion in the *MYBPC3* gene in human zygotes. Heterozygous carrier sperms were used to introduce this mutation. The wild type allele was provided by healthy donor oocytes. Preselected CRISPR – cas9 constructs had high specificity and targeting efficiency. The double stranded breaks in the mutant paternal *MYBPC3* gene were preferentially repaired using the wild-type oocyte allele as a template, suggesting an alternative, germline specific DNA repair response. Mechanisms responsible for mosaicism in embryos were also investigated and the authors proposed a solution to minimize their occurrence by the co-injection of sperm and CRISPR–Cas9 components into metaphase II oocytes.

Polyglutamine disorders: is therapy still a pipe dream? (Yang et al, 2017)

Huntington disease (HD) is one among the nine CAG/glutamine expansion neurodegenerative disorders, caused by CAG repeats in the *HTT* gene. These CAG repeats code for the polyglutamine (polyQ) tract in N terminal region of huntingtin and cause widespread cellular dysfunction. Previously siRNAs and antisense oligonucleotides, which rely on SNPs (Single Nucleotide polymorphisms) specific to mutant alleles, have been successfully shown to have a therapeutic effect in HD mice by suppressing the expression of transgenic mutant huntingtin (Carroll et al, 2011). In this study, investigators have tried permanent suppression of endogenous mHTT expression in the striatum of mHTT-expressing mice (HD140Q-knockin mice) using CRISPR/Cas9-mediated inactivation. They have demonstrated that this method effectively depleted HTT aggregates, atten-

uated early neuropathology and decreased motor deficits without affecting the viability of neurons. This study suggests that non–allele-specific CRISPR/Cas9-mediated gene editing could be used to efficiently and permanently eliminate polyglutamine expansion– mediated neuronal toxicity in the adult brain. This knowledge can be utilized as a therapeutic strategy in other CAG/glutamine expansion neurodegenerative disorders also.

Hitting the target in Duchenne muscular dystrophy (Bengtsson et al, 2017)

Duchenne muscular dystrophy (DMD) is an X linked disorder caused due to mutation in the dystrophin gene (DMD) which is a large gene with 79 exons. Systemic gene delivery via the vasculature by Adeno Associated Viruses (AAV) has been tried as a treatment strategy for DMD. Two promising methods tried using AAV were delivery of microdystrophins and direct gene editing using CRISPR/cas9. Gene editing using CRISPR/cas 9 was tried previously in induced pluripotent cells and murine germ cells. In vivo excision of exon 23 in murine Dmd gene was tried in mdx^{5cSn} mice model recently. Bengtsson et al. attempted to explore multiple gene editing strategies in the mdx^{4cv} mice model that harbor a nonsense mutation within exon 53, which is in the mutational hotspot region found in around 60% of DMD patients with deletion mutations. The authors developed and assessed multiple muscle-specific, AAV-CRISPR/Cas9-driven gene editing strategies for correction of the Dmd gene in dystrophic mdx^{4cv} mice. By both local and systemic delivery, dystrophin expression was widespread and there was a significant amelioration of the phenotype. The results indicate that AAV-CRISPR/Cas9-mediated gene editing has significant potential for the development of future therapies for DMD.

References

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