GeNeXpreSS

Genome editing technologies: Future of functional and therapeutic genetics!

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Minimal risk of miscarriage by invasive prenatal testing: Review and meta-analysis[1,2]

All over the world, the procedure related risk of miscarriage following an invasive prenatal testing is quoted to be 1-2%. Akolekar *et al.* have performed a meta-analysis of articles available on MEDLINE, EMBASE, CINHAL and Cochrane library in the period between 2000 and 2014.[1] The weighted pooled risk was estimated by 324 losses in 42,716 women undergoing amniocentesis and 207 losses in 8,899 women undergoing chorionic villous sampling. The authors concluded that the risk of procedure related risk of miscarriage after amniocentesis is 1:1000 and 1:500 after chorionic villous sampling which is not significantly different from the miscarriage rate in women who have not undergone the invasive testing. These figures can be used for counseling women who opt for invasive prenatal testing and help them in make informed choices.

Private mutations to private gene therapy: Beta thalassemia as an example[2]

Beta thalassemia major is one of the most common monogenic disorders worldwide. Hematopoietic stem cell transplantation from a histocompatible donor is the mainstay of therapy. However correction of mutation in the human beta globin gene (*HBB*) will be the ideal situation. Though few successful experiments of gene transfer using viral vectors have been performed, they have their own disadvantages including random insertion in the genome and the potential of insertional mutagenesis. Recently Xie *et al.* created induced pluoripotent stem cells from somatic cells of patient with beta thalassemia major harbouring compound heterozygous mutations in HBB gene.[2] These mutations in iPSCs were corrected using CRISPR/Cas9 system along with the piggybac system leading to a global switch to the normal gene and preserving the normal promoter sequences. The future in vivo use of this genome editing technology can lead to mutation specific gene therapy for the patients.

Restoring the reading frame in more than half of DMD patients: genome editing technology[3]

Various cell and gene based therapies are in preclinical/phase I trials for Duchenne muscular dystrophy (DMD). In majority of the patients, the disease is caused by deletion mutations which cause a shift in the reading frame, which leads to dysfunctional dystrophin protein production. The milder phenotype (Becker muscular dystrophy) is caused by mutations in the same gene, but these mutations are in-frame, which result in the formation of an abnormal but functional protein. Skipping of exon 51 by oligonucleotide-based therapy to restore the reading frame, has the potential to be used in 13% of DMD patients. If skipping of multiple exons is performed between 45-55 exons, which is the mutational hotspot, about 60% of all DMD patients can be offered therapy. However designing, the short half life and requirement of life-long injections of oligonucleotides are limiting factors. Recently Ousterout *et al.* have used CRISPR/Cas9 based genome editing technology to generate a 336 kb deletion across the 45-55 exons of dystrophin gene in skeletal myoblasts taken from DMD patients.[3] These edited cells were grafted in immunodeficient mice and expression of dystrophin protein was

observed. This study is a proof of the concept that CRISPR/Cas9 technology, being very versatile, can be used to correct mutations in 60% of DMD patients.

Promising curative therapy for HIV by genome editing technology: More than 30 years after first case of AIDS[4,5]

The first case of AIDS was described in 1981. Since then various forms of antiretroviral therapy (ART) are the main-stay of treatment. But antiretroviral treatment needs to be continued lifelong and is associated with significant side effects. CCR5 receptor is established to be important in entry of the HIV1 virus in CD4+ positive human T cells. Persons homozygous for a 32 base pair deletion in CCR5 (CCRΔ32) gene are naturally resistant to HIV1 virus and allogenic bone marrow transplantation from a homozygous CCRΔ32 donor is also shown to be curable in patients with AIDS. In recent years, knock down of CCR5 gene by using various technologies including ribozymes, short interfering RNA (SiRNA) and Zinc finger nuclease have been shown to be promising in acquiring resistance for HIV1 infection. CRISPR/Cas9 technology being rapid, efficient and high throughput, offers many advantages over existing genome editing technologies. Wang *et al.* have constructed a lentivirus vector coexpressing single guide RNA and Cas9 targeting CCR5 and transduced human CD4+ cells and showed the high frequency of CCR5 disruption, minimal off-target effects and stable transduction in cell lines.[4]

Another approach which is being explored is the eradication of the integrated HIV viral genome in CD4+ cells which subsequently cause viral reactivation and viremia as soon as 50 days of stopping the ART. This integrated viral genome is not treatable by existing ART. Strong *et al.* have used transcription activator like effector nucleases (TALENs) to show that this genome editing technology can cleave integrated viral genome with high specificity and efficiency in vitro and in living cells.[5] The authors also constructed the TALEN variants which can recognise wild type and triple mutant viral sequences which can escape from other genome editing technologies included CRISPR/Cas9.

Together these experiments raise the hope for effective curative treatment to be available for patients infected with HIV1.

References

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