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In vitro Modeling of Aniridia-Related Keratopathy by the Use of Crispr/Cas9 on Limbal Epithelial Cells and Rescue

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Abstract : Haploinsufficiency of PAX6 in humans is the main cause of congenital aniridia, a rare eye disease characterized by reduced visual acuity. Patients have also progressive disorders including cataract, glaucoma and corneal abnormalities making their condition very challenging to manage. Aniridia-related keratopathy (ARK), caused by a combination of factors including limbal stem-cell deficiency, impaired healing response, abnormal differentiation, and infiltration of conjunctival cells onto the corneal surface, affects up to 95% of patients. It usually begins in the first decade of life resulting in recurrent corneal erosions, sub-epithelial fibrosis with corneal decompensation and opacification. Unfortunately, current treatment options for aniridia patients are currently limited. Although animal models partially recapitulate this disease, there is no in vitro cellular model of AKT needed for drug/therapeutic tools screening and validation. We used genome editing (CRISPR/Cas9 technology) to introduce a nonsense mutation found in patients into one allele of the PAX6 gene into limbal stem cells. Resulting mutated clones, expressing half of the amount of PAX6 protein and thus representative of haploinsufficiency were further characterized. Sequencing analysis showed that no off-target mutations were induced. The mutated cells displayed reduced cell proliferation and cell migration but enhanced cell adhesion. Known PAX6 targets expression was also reduced. Remarkably, addition of soluble recombinant PAX6 protein into the culture medium was sufficient to activate endogenous PAX6 gene and, as a consequence, rescue the phenotype. It strongly suggests that our in vitro model recapitulates well the epithelial defect and becomes a powerful tool to identify drugs that could rescue the corneal defect in patients. Furthermore, we demonstrate that the homeotic transcription factor Pax6 is able to be uptake naturally by recipient cells to function into the nucleus.

Keywords : Pax6, crispr/cas9, limbal stem cells, aniridia, gene therapy
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