Small-activating RNA therapy development for the inherited genetic disorder Aniridia

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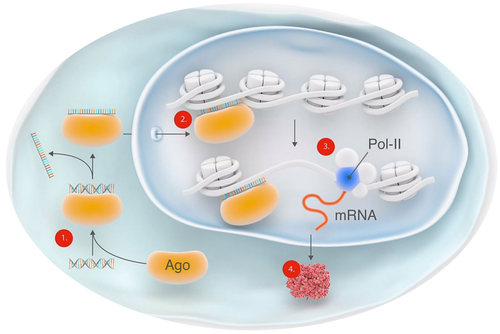
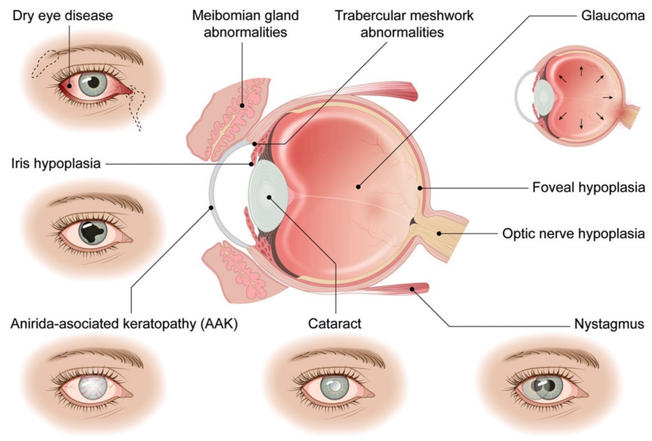
Background

Small activating RNAs (saRNAs) are synthetic 21-nucleotide double-stranded RNAs. They bind to promoters at the DNA level to increase transcriptional activation within physiological limits. saRNAs have already shown promise in clinical trials of cancer treatment; however, their potential for inherited diseases remains underexplored





Aniridia is a rare inherited eye disease caused by a range of mutations in the transcription factor PAX6, that lead to loss of function of one allele and ~50% pax6 protein expression.

Current treatments provide temporary relief to the symptoms but do not address the cause of the disease.

As the majority of aniridia patients have one fully functional and one non-functional gene copy, upregulation of the functional copy may alleviate the symptoms and/or prevent the progression of the disorder.

Our aim is to develop a saRNA that upregulates PAX6 to physiological levels.

Results

1. Identification and validation of a lead saRNA in multiple corneal cell lines

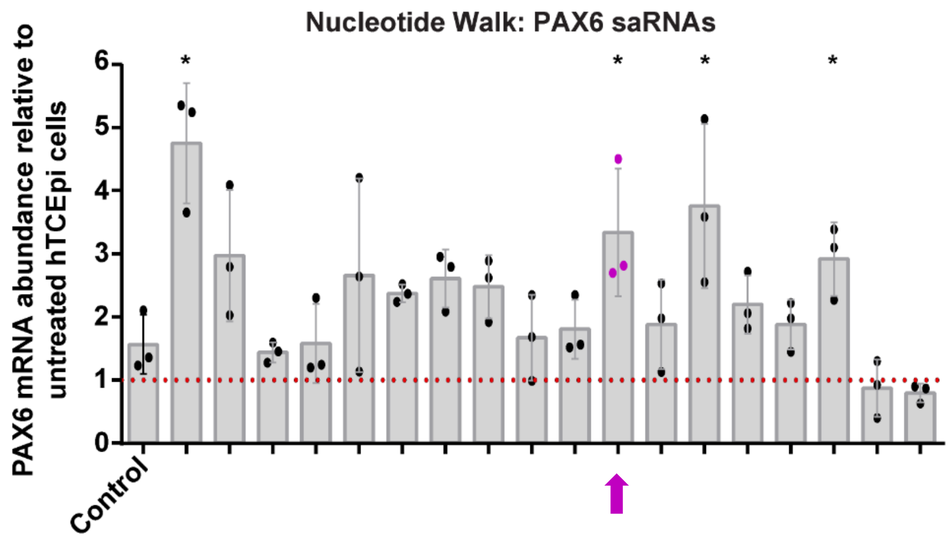


Figure 1: RT-qPCR analysis of human limbal-derived corneal epithelial cells (hTCEpi) transfected with a panel of saRNAs. Relative abundance of PAX6 normalised to the geometric mean of three reference transcripts was determined 24 h after transfection. Each point represents an independent experimental repeat plotted as fold change relative to untreated hTCEpi cells. * denotes statistical significance at p<0.05 compared with untreated cells by one-way ANOVA with Tukey's post-hoc test.

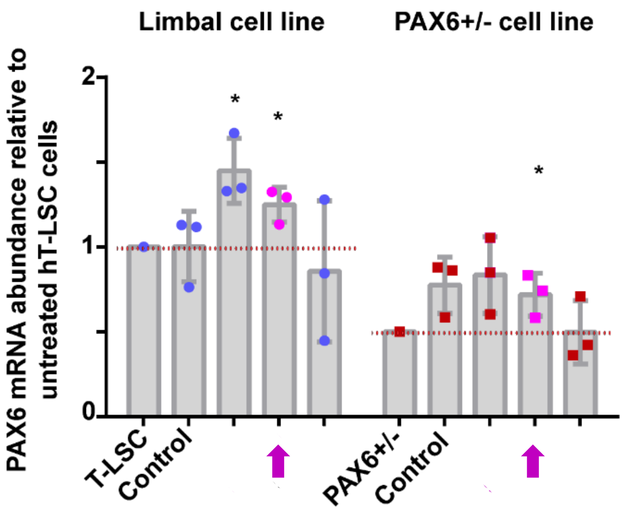


Figure 2: saRNAs were tested in human limbal-derived corneal epithelial cell line (T-LSCs) and in PAX6 +/- haploinsufficient T-LSC cells. Each point represents an independent experimental repeat. * denotes statistical significance at p<0.05 compared with untreated cells by one-way ANOVA with Tukey's post-hoc test.

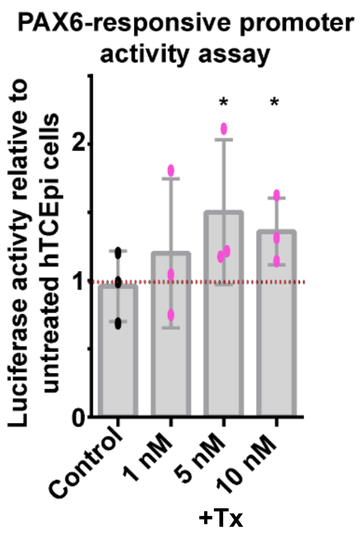


Figure 3: **hTCEpi cells transfected with control saRNA at 10 nM or lead saRNA (Tx) at 1, 5 or 10 nM, transfected with a plasmid encoding a luciferase reporter driven by a PAX6 response DNA element**. Luciferase activity was measured and plotted relative to untreated hTCEPi cells with each point representing an individual experiment. * denotes p<0.05 for differences from control-saRNA treated cells.

2. Treatment with lead saRNA rescues PAX6 gene targets in PAX6 +/- cell line

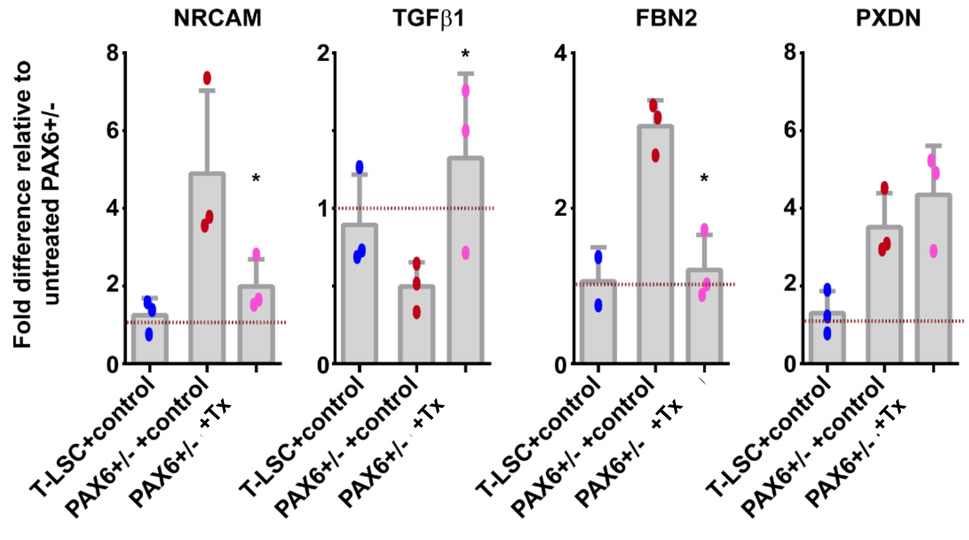


Figure 4: Relative expression levels of PAX6 target genes in T-LSC line (red dotted line), T-LSCs transfected with control saRNA (blue dots), in PAX6+/- cells transfected with control saRNAs (PAX6+/-, red dots) or in PAX6+/- cells transfected with lead saRNA (Tx) (magenta dots). RT-qPCR was performed for the indicated genes with comparisons normalised to the geometric mean of three reference transcripts. * denotes statistically significant difference compared with untreated PAX6+/- cells.

3. Lead saRNA rescues PAX6 +/- cell scratch wound and adhesion defects

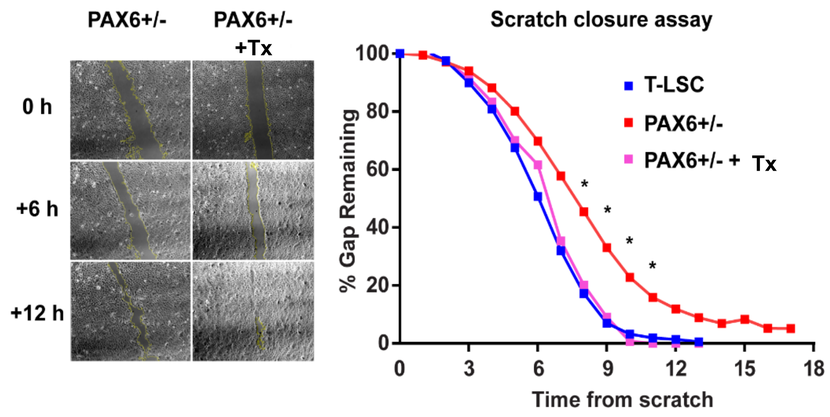


Figure 5: In vitro scratch assay comparing T-LSC (blue), with PAX6+/- cells (red) and PAX6+/- transfected with 10 nM lead saRNA (Tx) (magenta). Lines represent the mean of three independent experiments

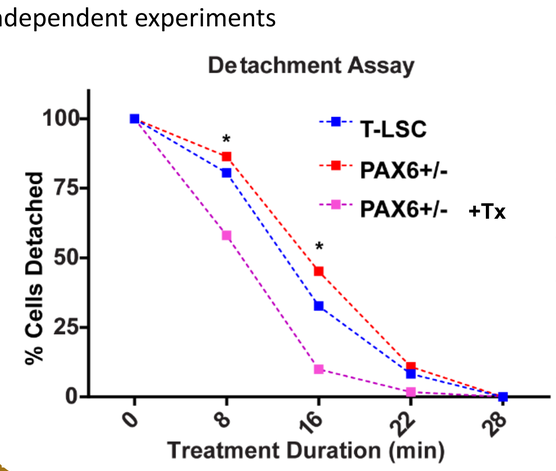


Figure 6: In vitro cell detachment assay comparing T-LSC (blue), with PAX6+/- cells (red) and PAX6+/- cell transfected with 10 nM lead saRNA (Tx) (magenta). Cells were plated on uncoated tissue culture plastic dishes then treated with a dilute trypsin solution to dissociate the cells. Samples were taken at the indicated time points and counted, then plotted relative to the total cell count as the mean from three independent experiments. * denotes statistically significant differences between PAX6+/- cells and PAX6+/- cells treated with lead saRNA (Tx) p<0.05.

Conclusion

A lead saRNA was identified and, importantly, treatment with the lead rescued aniridia-mimicking phenotypic aspects of the *PAX6* +/- line—i.e., rescuing downstream gene expression levels, cell motility, and the cell adhesion defects of the mutant cell line. Together these positive data provide a solid basis for onward development of a saRNA based treatment for aniridia and other PAX6-related disorders.