Bioinformatic analysis reveals possible mechanisms of aniridic keratopathy

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Abstract

PAX6 is a master regulator of eye development expressed in the eye field of the head ectoderm which gives rise to both the corneal and conjunctival epithelium in adults. Homozygous PAX6 mutations are embryonic lethal while heterozygous PAX6 mutations lead to Aniridia syndrome in humans, and the Small eye (SEY) phenotype in mice. In Aniridia, the cornea appears normal at birth, but often becomes opaque and vascularized with age, a condition defined as Aniridic keratopathy (ARK). While the mechanisms underlying ARK are unclear, these are proposed to include invasion of the corneal surface with cells from the adjacent conjunctiva. To test the possible mechanisms driving ARK, we took an unbiased approach and performed RNA-seq analysis on corneas from 20-week-old PAX6 +/- mice and their wildtype littermates which revealed that 823 genes were differentially expressed in the Pax6 +/- cornea, with 514 upregulated and 309 downregulated. These data revealed an upregulation of the conjunctival cytokeratins-13, 15 and 19, and downregulation of the corneal cytokeratin, keratin 12, which aligns with the concept that the cornea epithelium is being invaded by conjunctiva. To further explore this hypothesis, we compared this DEG list with a prior study that identified genes differentially expressed between the human cornea and conjunctiva (GEO Accession GSE38190). This analysis found that 157 of the genes whose expression is altered in the SEY cornea exhibited either cornea or conjunctiva preferred expression. Of these, 95 were upregulated in the PAX6+/- cornea and exhibited conjunctiva preferred expression in human eye while 33 genes were downregulated in the SEY cornea and normally exhibit corneal preferred expression in humans. These results are consistent with the proposition that ARK, at least in part, results from conjunctivalization of the cornea.

Aniridia and Aniridic Keratopathy

- Aniridia is a rare congenital autosomal dominant, bilateral, panocular condition that is characterized by iris hypoplasia, glaucoma, nystagmus, foveal hypoplasia, ultimately leading to blindness.
- Haploinsufficiency of the PAX6 transcription factor resulting from heterozygous mutation of the PAX6 gene is the major cause of Aniridia.
- A major negative consequence to vision is Aniridia-related Keratopathy that includes limbal stem cell deficiency and conjunctivalization.

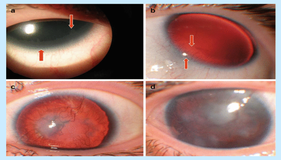


Figure 1. Phases of Aniridia Related Keratopathy [1].

(A)- Thickening of corneal epithelium.
(B)- Superficial vascularization extending to central cornea.
(C) and (D)- Neovascularization and extension of keratopathy to whole cornea with stromal opacifications.

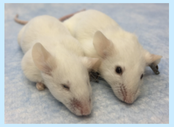
- Limbal Stem Cells (LSC) reside at the cornea-scleral junction in an area known as limbus. LSC promote corneal would healing and corneal epithelial cell replenishment.
- Limbal Stem cell deficiency is associated with conjunctivalization, neovascularization, inflammation and fibrotic lesions which are otherwise clear and avascular.
- However, the primary mechanisms leading to aniridic phenotypes are still unknown.

To understand the possible mechanisms underlying aniridia using an unbiased approach, RNA-SEQ was performed on PAX +/- mice.

Mouse Aniridia Model *Pax6*$^{+/tm1Pgr}$

- The *Pax6*$^{+/tm1Pgr}$ mouse with only one functional Pax6 allele resembled human aniridic model.
- They have a specific phenotype of smaller sized eye and known as Small Eye mice (SEY).

Figure 2: SEY mouse showing the small eye phenotype and a wildtype littermate with normal sized eyes.



RNA-Seq Analysis

- RNA-Seq analysis was performed on corneas from 20-week old Pax6$^{+/tm1Pgr}$ and their wildtype littermates.
- 823 genes were differentially expressed with 514 upregulated and 309 downregulated.
- An upregulation of the conjunctival marker-Krt13 and downregulation of the corneal marker-Krt12 in the mutant corneas revealed a conjunctival phenotype of the cornea aligning with human studies that showed a conjunctival phenotype of the aniridic corneas suggesting SEY mice to be a model for human aniridia.

Pax6 Mutant Corneas Express Conjunctiva Preferred Genes as well as Inflammatory and Angiogenic markers

- DE statistics were calculated, and intersecting genes were evaluated from the GSE38190 series-matrix, that compares human cornea and conjunctiva, mapped human to mouse homologs using a table from MGI.

	Mutant vs Wildtype Cornea		Conjunctiva vs. Cornea	
Gene Symbol	Fold Change	FDR p value	Fold Change	FDR adjusted p value
Inflammatory Marker:				
Il33	17.51	0.0004	4.47	0.0004
Fibrotic Marker				
Tnc	10.49	0.0012	0.67	0.47
Conjunctival Marker:				
Krt13	20.4	2.81E-05	5.75	0.0002
Krt19	5.26	0.0025	4.79	0.002
Osr2	7.31	0.0005	7.28	0.00002
Corneal Marker:				
Krt12	-3.5	0.0029	-3.79	0.228
Angiogenesis Marker				
Vegfa	2.39	0.003	2.08	0.04

Table 1. Comparative differential expression of selected genes from both Pax6 Mutant vs Wildtype Cornea; Conjunctiva vs Cornea.

Corneal to Conjunctival Transition in Pax6 mutant Mice

Figure 3: Intersection between Pax6 dependent corneal genes and genes that are conjunctival or corneal preferred. Total (A), up (B) and down (C) regulated.

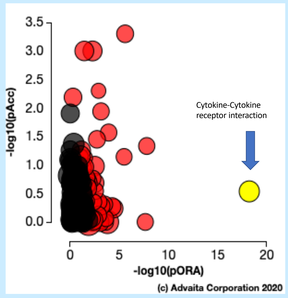


Figure 4: Plot showing the Cytokine-cytokine receptor interaction

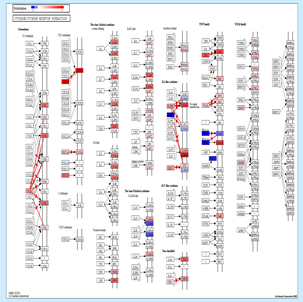


Figure 5: Cytokine-Cytokine receptor interaction pathway in Mutant vs. Wildtype Corneas

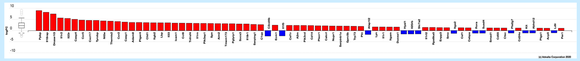


Figure 6 : Plot showing the inflammatory genes that were differentially expressed in the Mutant vs. Wildtype Corneas.

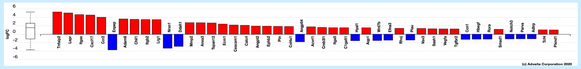


Figure 7: A plot showing the angiogenesis genes that were differentially expressed in the Mutant vs. Wildtype corneas.

Conclusion

- Conjunctivalization of PAX6 mutant corneas.
- Upregulation of the Cytokine-cytokine interaction pathway can be linked to increased angiogenesis, cell proliferation in response to inflammation and wound healing.
- PAX6 mutant mice as animal models of Aniridia

References

1. García J.S.L., Lozano I.G. (2015) Aniridic Keratopathy: Conservative Approaches. In: Parekh M., Poli B., Ferrari S., Teofili C., Ponzin D. (eds) Aniridia. Springer, Cham

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