Single-Cell Transcriptomics of the TAZ-deficient Murine Cornea

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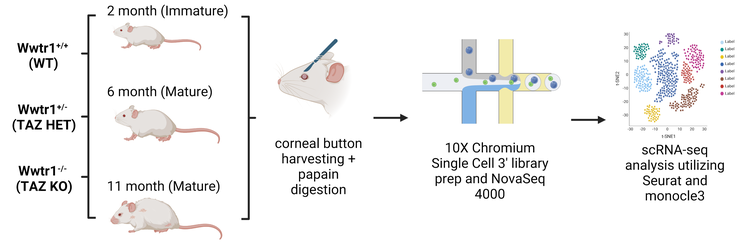
INTRODUCTION

Fuchs' endothelial corneal dystrophy (FECD) is a polygenic disease affecting >300 million individuals globally under the age of 30 [1]. The disease is characterized by accelerated and progressive loss of corneal endothelial cells (CEnC) with degenerative processes influencing the Descemet membrane (DM) such as accumulation of aberrant extracellular matrix (ECM) and guttae formation [2]. Patients develop corneal edema and consequent vision loss requiring transplantation. However, the lack of donor tissue in some regions of the world as well as surgical complications warrant identification of novel therapeutics.

The late-onset form of the disease can be modeled in mice that are deficient in *Wwtr1*, a gene that encodes the transcriptional co-activator with PDZ-binding motif (TAZ) [3]. TAZ is not only a mechanotransducer of matrix stiffness and geometry but a central orchestrator of multiple signaling pathways key to the maintenance of CEnC and limbal stem cell (LSC) health as well as those implicated in FECD such as Hippo, FGF, TGF-Beta and NF-kB [4–10]. Thus, this project aims to characterize how *Wwtr1* (TAZ) deficiency mechanistically contribute to the pathogenesis of FECD in CEnC and to determine whether there is an impact on the LSC and consequently the corneal epithelium (CEp).

METHODS



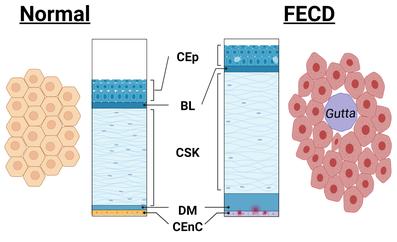
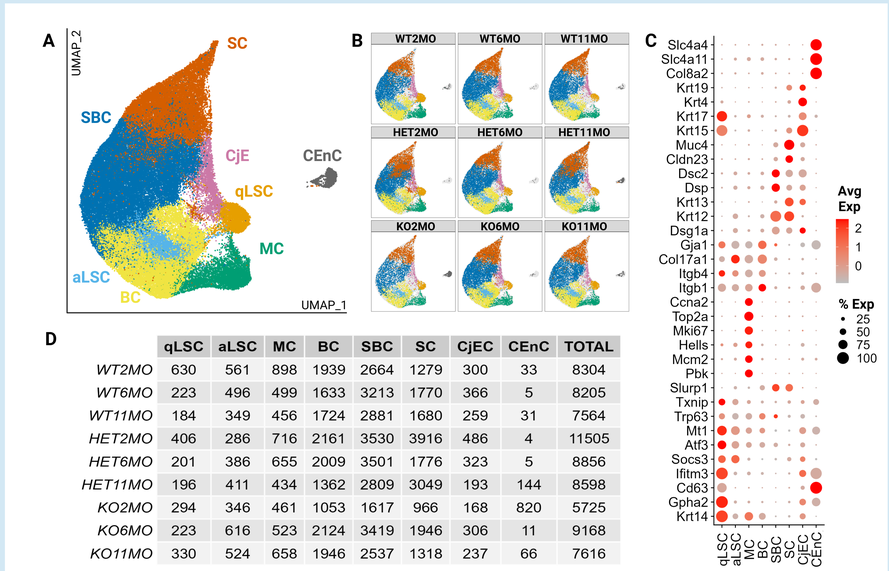


Fig 1. The schematic diagram of a normal versus FECD cornea. In the FECD cornea, there is an increased rate of CEnC attrition, abnormal CEnC morphology, cloudy stroma (CSK) and Descemet membrane (DM) thickness and stiffness alterations. No significant changes noted in the corneal epithelium (CEp) and the Bowman's layer (BL).

RESULTS



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	qLSC	aLSC	MC	BC	SBC	SC	CjEC	CEnC	TOTAL
WT2MO	630	561	898	1939	2664	1279	300	33	8304
WT6MO	223	496	499	1633	3213	1770	366	5	8205
WT11MO	184	349	456	1724	2881	1680	259	31	7564
HET2MO	406	286	716	2161	3530	3916	486	4	11505
HET6MO	201	386	655	2009	3501	1776	323	5	8856
HET11MO	196	411	434	1362	2809	3049	193	144	8598
KO2MO	294	346	461	1053	1617	966	168	820	5725
KO6MO	223	616	523	2124	3419	1946	306	11	9168
KO11MO	330	524	658	1946	2537	1318	237	66	7616

Fig 3. Single-cell atlas of the murine cornea demonstrates the presence of 9 cell types across all three genotypes of the three ages. (A) UMAP of the scRNA-seq atlas across 9 samples (n = 27). qLSC, quiescent limbal stem cells; aLSC, active limbal stem cells; MC, mitotically active cells; BC, basal cells; SBC, suprabasal cells; SC, superficial cells; CjEC, conjunctival epithelium; CEnC, corneal endothelial cells. (B) UMAP of single cell atlas split by sample and colored by cell type. (C) Dot plot demonstrating cell type specific marker expression [11-13]. (D) Table of cell type frequencies across samples.

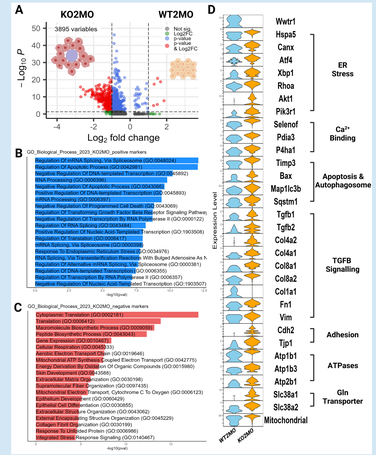

Fig 4. CEnC from TAZ KO demonstrate an upregulation of transcripts associated with endoplasmic reticulum (ER) stress, cell death and mitochondrial dysfunction compared to WT CEnC at 2 months of age. (A) Volcano plot demonstrating differentially expressed (DE) genes between KO2MO (n = 820) and WT2MO (n = 33) CEnC. $p < 0.05$ (B) Bar chart demonstrating the top 20 gene ontology hits for biological processes for genes upregulated by CEnC KO2MO with respect to WT2MO. (C) Bar chart demonstrating the top 20 gene ontology hits for biological processes for genes downregulated by CEnC KO2MO with respect to WT2MO. (D) Violin plot of select DE genes of biological significance. $p < 0.05$.

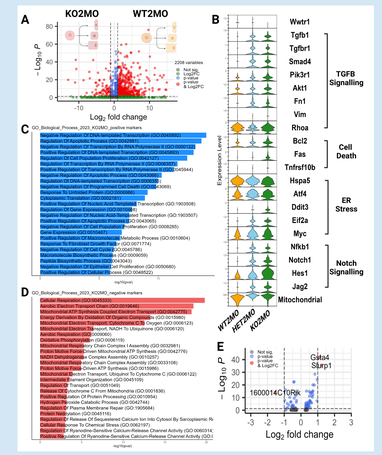

Fig 5. qLSC from TAZ KO demonstrate an upregulation of transcripts associated with the TGF-B pathway and Notch pathway compared to WT CEnC which does not persist with age. (A) Volcano plot demonstrating DE genes between KO2MO (n = 294) and WT2MO (n = 630) qLSC. $p < 0.05$ (B) Bar chart demonstrating the top 20 gene ontology hits for biological processes for genes upregulated by qLSC KO2MO with respect to WT2MO. (C) Bar chart demonstrating the top 20 gene ontology hits for biological processes for genes downregulated by qLSC KO2MO with respect to WT2MO. (D) Violin plot of select DE genes of biological significance. $p < 0.05$. (E) Volcano plot demonstrating DE genes between KO11MO (n = 330) and WT11MO (n = 184) qLSC. $p < 0.05$.

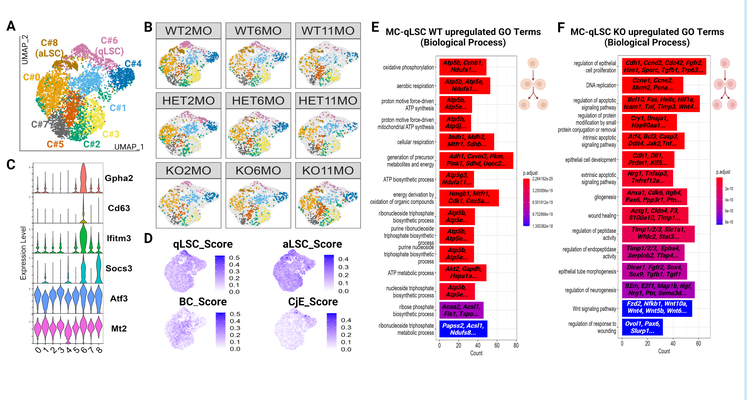

Fig 6. TAZ KO mitotically active qLSC (MC-qLSC) exhibit a greater degree of "stemness" and proliferation than their WT counterparts. Unbiased clustering of MC reveals 8 subtypes. (A) UMAP of scRNA-seq atlas of MC across 9 samples (n = 27). (B) UMAP of MC split by sample and colored by MC subtype. (C) Violin plot of qLSC (*Gpha2, Cd63, Ifitm3*) and aLSC markers (*Socs3, Atf3, Mt2*). (D) Dim plots of composite scores of the signatures of the 4 proliferating cell types of the cornea. Cell type scores were determined using the top 10 markers of the 4 cell type clusters. (E,F) Bar chart demonstrating the top 15 gene ontology (GO) hits for biological processes for genes upregulated between age matched WT MC-qLSC and KO MC-qLSC, respectively. Sample upregulated genes from each GO category are listed.

CONCLUSION

- Established an updated cell atlas with scRNA-seq of the murine cornea with age and genotype (TAZ deficiency)
- KO2MO CEnC have an upregulation of ER stress pathways, calcium binding proteins, cell adhesion and cell death with respect to WT2MO CEnC.
- KO2MO CEnC also exhibits a compensatory increase in expression of cell adhesion molecules, Na/K ATPases and mitochondrial genes.
- Non-proliferating KO2MO qLSC upregulated TGF-Beta and Notch signaling pathways but downregulated genes associated with aerobic respiration compared to WT2MO.
- Non-proliferating qLSC has much fewer DE genes at the 11-month age between genotypes.
- KO MC-qLSC downregulates aerobic respiratory pathways but increase pathways of proliferation and stem-cell maintenance compared to age matched WT MC-qLSC.

REFERENCES

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