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A single cell atlas of developing and adult cornea in steady state and disease conditions

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Purpose : Single cell (sc) analyses of key embryonic, fetal and adult stages were performed in order to generate the first comprehensive single cell atlas of all the corneal and adjacent conjunctival cell types from development to adulthood.

Methods : Four human adult and seventeen embryonic and fetal corneas from 10-21 post conception week (PCW) specimens were dissociated to single cells and subjected to scRNA- and/or ATAC-Seq using the 10x Genomics platform.

Results : scRNA-Seq analysis of 21,343 cells from four adult human corneas and adjacent conjunctivas revealed the presence of 21 cell clusters, representing the stem, progenitor and differentiated cells in all layers of cornea and conjunctiva as well as immune cells, melanocytes, fibroblasts and blood/lymphatic vessels. A small cell cluster with high expression of limbal stem cells (LSCs) markers was identified and shown via pseudotime analysis to give rise to five other cell types representing the progenitor and differentiated corneal epithelial cells. A novel putative LSCs surface marker, GPHA2, expressed in 0.41% ± 0.21 of the cultured limbal epithelial cells, was identified, based on predominant expression in the limbal crypts of adult and developing cornea and RNAi validation in cultured LECs. Combining scRNA- and ATAC-Seq analyses, we identified multiple upstream regulators for LSCs and transit amplifying (TA) cells and demonstrated a close interaction between the immune cells and corneal epithelial stem and progenitor cells. RNA-Seq analysis indicated the loss of LSCs markers and acquisition of proliferative limbal basal epithelial progenitor markers during *ex vivo* limbal epithelial cell expansion, independently of the culture method used. Single cell RNA-Seq of 89,897 cells obtained from embryonic and fetal cornea indicated that during development, the conjunctival epithelium is the first to be specified from the ocular surface epithelium, followed by the corneal epithelium and the establishment of LSCs/progenitor cells.

Conclusions : Our scRNA-and ATAC-Seq data of developing and adult cornea in steady state and disease conditions provide a unique resource for defining genes/pathways that can lead to improvement in *ex vivo* LSCs expansion, stem cell differentiation methods and better understanding and treatment of ocular surface disorders.