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Human lens epithelial cells induce the inflammatory response when placed into the lens capsular bag model of posterior capsular opacification

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Purpose: Cataracts are typically treated by phacoemulsification followed by intraocular lens implantation. Studies of mouse models of cataract surgery have revealed that lens epithelial cells rapidly remodel their transcriptome to express proinflammatory cytokines after lens fiber cell removal, but it is currently unknown whether this response is conserved in human lenses. This study seeks to fill this knowledge gap.

Methods: Human cadaver eyes from 70 to 89 year old individuals were prepared for the human capsular bag model of cataract surgery. The central epithelium was preserved in RNAlater during culture preparation, then the equatorial epithelium was either immediately preserved in RNAlater after the culture was created, or 24 h later. Gene expression profiles were generated by bulk sequencing of RNA isolated from these tissue samples. The transcriptomic response of human cadaver-derived lens epithelial cells to culture in this "capsular bag" model was characterized by bioinformatic analysis. The human response was directly compared to that of 24-month-old mouse lens epithelial cells subjected to fiber cell removal surgery.

Results: Human lens epithelial cells remodel approximately a third of their transcriptome by 24 h after surgery, and like mice, this response consists of induction of proinflammatory cytokine genes, upregulation of fibrotic markers and downregulation of genes controlling the lens epithelial phenotype.

Conclusions: These observations demonstrate that humans and mice have similar responses to cataract surgery and support the use of mice to study the response of lens epithelial cells to cataract surgery, suggesting that identified injury response mechanisms can be leveraged to elucidate new approaches to improve the outcomes of cataract surgery.

Cataract, defined as the clouding of the ocular lens, has historically been a major cause of human blindness. However, the incidence of cataract-induced visual disability has greatly decreased in recent decades due to the development and refinement of surgical cataract treatment combined with its increased global availability [1,2]. Phacoemulsification, the most common method used today, removes the central lens epithelium/capsule and lens fibers followed by placement of an intraocular lens (IOL) prostheses into the retained lens capsule to restore refraction [3,4].

For most patients, cataract surgery is highly successful, restoring vision while improving their overall quality of life [5]. However, like all surgeries, cataract surgery is not completely benign as it often induces acute ocular inflammation [6] which can precipitate vision compromising macular edema [7] and in rare cases, retinal detachment [8,9]. Further, the most common cataract surgery methods do not remove all of the lens epithelial cells (LECs) from the retained peripheral anterior lens capsule, and these cells undergo a wound healing response which causes them to proliferate, migrate across the denuded lens capsule, and differentiate into a mixture of dysmorphic lens fiber cells and myofibroblasts which disrupt vision if they reach the visual axis, a condition called posterior capsular opacification (PCO) [10,11].

It is recognized that transforming growth factor-beta (TGF-β) signaling in lens capsule associated cells (CACs) is critical for myofibroblast formation [12,13] and persistence [14] post cataract surgery (PCS), however much less is known about the mechanisms by which cataract surgery triggers this signaling. Notably, we have discovered that it takes 48 to 72 h for the canonical TGF-β pathway, as measured by elevations in pSMAD2/3 levels, to activate in CACs PCS [15], likely due to the need to upregulate the expression of αVβ8-integrin, an important regulator of latent TGF-β activation [16]. To understand how cataract surgery induces this pathway, we performed RNAseq profiling on CACs collected at 24 h following lens fiber cell removal in a mouse model which unexpectedly revealed that a large proportion of the LEC

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transcriptome was remodeled by 24 h PCS [15], a response which included the downregulation of many genes regulating the lens epithelial phenotype and induction of proinflammatory cytokine and fibrotic marker expression [15,17].

As human LECs also produce growth factors and inflammatory cytokines when cultured in vitro in the capsular bag model of PCO [18], and the aqueous humor of cataract patients exhibits highly elevated levels of inflammatory cytokines by 20 h PCS [19], it is possible that the LECs remaining behind PCS could play a direct role in driving ocular inflammation, i.e., flare, PCS [20]. Further, as inflammation can drive fibrotic disease in other tissues [21,22], this result suggests that the early inflammatory response of LECs to cataract surgery could set the stage for their later conversion to myofibroblasts, thus leading to fibrotic PCO.

While the mouse has been long used as an animal model of cataract [23-25], mouse and human lenses do exhibit structural and molecular differences [26-28], although the similarities between the molecular responses to injury/surgery in these species have not been comprehensively evaluated using unbiased approaches. Here we perform bulk RNAseq profiling of the central and equatorial lens epithelium isolated from human cadaver lenses obtained from elderly donors and compared the resulting transcriptomes with our prior transcriptomic profiling of aged mouse lens epithelial cells [17]. The acute response of human equatorial LECs to the lens capsular bag model of cataract surgery [29] was assessed by transcriptome profiling of cells obtained either immediately after explant creation or following 24 h of culture. These data provide a comprehensive overview of the similarities and differences between the mouse and human LEC transcriptome and confirm that the mouse is a valid model to understand the acute response of LECs to cataract surgery.

METHODS

Human tissue: Aged human donor eye globes (70–80 years of age) were obtained from the "Banc d´Ulls per a Tractaments de Ceguesa" eye bank (Barcelona, Spain) in concordance with the Tenets of the Declaration of Helsinki. The experimental protocol was approved by the Ethical Committee for Clinical Research of the Centro de Oftalmología Barraquer. Age, cause of death, sex, and post mortem time of each donor can be found in Appendix 1.

Lens capsular bag cultures: Human lens capsular bag cultures were created as previously described with all steps performed at room temperature [29,30]. Briefly, the corneoscleral disk was removed from the globe via a circular trephine and the iris-ciliary body-lens complex dissected, then transferred to a sterile Petri dish. Meanwhile, a silicone ring mount was placed in a Petri dish and immersed in Hanks balanced salt solution (HBSS) with added 2% antibiotic/antimycotic (10,000 U penicillin, 10 mg streptomycin, and 25 mg amphotericin B per milliliter). The iris-ciliary body lens complex was then placed on the silicone ring, anterior surface facing upward, attached by placing eight 30-gauge needles through the ciliary body and the iris removed. A capsulorhexis was then performed on the lens, with the anterior capsulotomy specimen (lens capsule with attached central epithelial cells) immediately collected in RNALater (Sigma-Aldrich, Madrid/Spain). The fiber cells were removed by hydrodissection and hydroexpression through the capsulorhexis and cortical fibers also placed in RNAlater. One capsular bag from each globe pair (lens capsule with attached equatorial epithelial cells) was then immediately separated from the zonules and placed in RNAlater, as a 0 h control. The fellow capsular bag was washed 3 times for three minutes each with HBSS and then cultured in a humidified CO_2-incubator at 37 C and 5% CO_2 with RPMI-1640 medium (supplemented with 5% fetal calf serum (FCS), 1% 10,000 units penicillin, 10 mg streptomycin and 25 µg amphotericin B per milliliter). After 24 h, the capsular bag (lens capsule with attached equatorial epithelial cells) was separated from the zonules and also submerged in RNAlater. The samples in RNAlater were then shipped to the University of Delaware for further analysis.

RNA extraction and RNAseq analysis: The capsular fragments with attached lens epithelial cells were removed from RNAlater, and RNA isolated using the RNeasy Micro Kit from Qiagen (Catalog Number: 74004). RNAseq libraries were prepared using the SMARTer® Stranded Total RNAseq Kit-Pico Input Mammalian (Takara Bio, Inc., Mountain View, CA) and sequenced by DNA Link (1000 S Hope St. unit 521 Los Angeles, CA 90015) on a Novaseq 6000 (San Diego, CA). This yielded approximately 35 million raw sequencing reads (paired end, 101 nucleotides long) per sample which were assessed for quality, and trimmed for adaptor content and low-quality base calls, using the Babraham Institute's "FastQC" and "Trim Galore!" programs.

As SMARTer® Stranded Pico RNAseq libraries often have significant numbers of reads derived from rRNA, trimmed paired end reads were first aligned to the human 45S pre-spliced rRNA transcript (RN45SN5), and reads aligning to this transcript excluded from downstream analyses. All remaining paired end reads of at least 85 nucleotides were aligned to the Ensembl primary assembly of the human GRCh38 genome [31] using HISAT2 with its default parameters [32]. Read pairs aligning to genomic features in the Ensembl Human version 104 GTF file were quantified as gene level counts, using HTSeq-Count in union mode [33].

Length normalized abundance estimates (Fragments per Kilobase-Million (FPKM)) were calculated from gene level counts using the total length of all known exons for a given gene, after merging overlapping exons. Genes with at least 10 mapped reads in at least four samples were considered to have "detectable" levels of expression, those failing criteria were removed before normalization and differential expression testing using edgeR's "filterByExpr" function [34]. Gene level counts were normalized, and pairwise differential gene expression statistics calculated using robust dispersion estimates with the "Trimmed Median of Means" (TMM) [35] and "exactTest" methods implemented in edgeR [36]. Biologically significant differentially expressed genes (DEGs) were defined as those exhibiting a statistically significant difference in expression using Storey's Q value to adjust for false discovery rate (Q ≤0.05) [37], a difference in expression level greater than 2 FPKM between conditions, Fold Change (FC) greater than 2 in either the positive or negative direction and expressed at a level greater than 2 FPKM [38]. Tables of differentially expressed genes (DEG Tables) were generated for the following pairwise contrasts: freshly isolated equatorial versus central epithelium, and freshly isolated equatorial epithelium versus equatorial epithelium cultured 24 h in the lens capsular bag model. The resulting FastQ files and analyzed data were deposited in the Gene expression omnibus (GEO) under Accession number GSE186716.

Pathway analyses: iPathwayGuide (Advaita Bioinformatics, Plymouth, MI) was used for pathway analysis performed on all statistically significant DEGs defined as those exhibiting a fold change ≥ 2 and false discovery rate corrected p value (FDR) ≤0.05. The iPathwayGuide uses Impact Analysis, which considers the directed interactions of DEGs within a pathway (as defined by the Kyoto Encyclopedia of Genes and Genomes [39] (KEGG, Release 96.0+11–21, Nov 20) and if more pathway participants are observed in the DEG list than would be estimated by chance [40]. Gene ontology comparisons were made against the October 14, 2020 release of the Gene Ontology Consortium database [41].

Comparison of gene expression in human and mouse lens epithelial cells: FastQ files generated from RNAseq data previously obtained from mouse lens epithelial cells isolated from 24-month-old mice subjected to the in vivo mouse model of cataract surgery [42] both the time of lens fiber cell removal and 24 h later [17] (Geo accession number GSE166619) were reanalyzed using the same pipeline as the human LEC samples above except that it was mapped to the mouse genome (Ensembl GRCm39, GTF v104) and the mouse Rn45s pre-spliced transcript used for ribosomal filtering. This resulted in expressed gene tables representing the naïve 24 month old mouse LEC transcriptome and the changes to this transcriptome that manifest 24 h after fiber cell removal surgery.

Ensembl's v104 homology mappings were used to identify mouse homologs of human genes. For genes where multiple homologs were detected, homolog pairs used for further analysis were chosen based on Ensembl orthology confidence score and percentage of sequence identity. Genes with recognized homologs in mouse and human were then ranked based on their group mean abundance (FPKM). For each cell type (naïve human central and equatorial epithelium, equatorial epithelium cultured for 24 h; mouse, naïve lens epithelium, lens epithelium 24 h after fiber cell removal), the genes with known homologs in the other species were rank ordered based on mean abundance, and then partitioned into three categories: only within the top 10% of abundance in human LECs, in the top 10% of abundance in both mouse and human LECs, and only in the top 10% of abundance in mouse LECs. Human central LEC and human equatorial LEC profiles were each compared to profiles generated from the entire mouse lens epithelium in separate analyses. The genes meeting differential expression criteria in the human and mouse comparisons were compared with the genes annotated to participate in either KEGG Pathway "Cytokine / Cytokine-Receptor Interaction" (KEGG ID: map04060) or a set of well characterized genes that are known to be associated with fibrosis and/or epithelial to mesenchymal transition (EMT; MSigDB Hallmark EMT) [43].

RESULTS

We previously reported that remnant lens epithelial cells (LECs) massively remodel their transcriptome by 24 h after lens fiber cell removal in a mouse model of cataract surgery [15-17,44]. The most impacted pathways were related to the inflammatory response, most notably, cytokine-cytokine receptor interactions, although the mRNA levels of key fibrotic markers are also upregulated by this time [15,17]. However, the relevance of these observations to the response of human LECs to cataract surgery, and the progression of post-surgical ocular inflammation in humans, was unclear as there are known species differences in lens gene expression between mouse and human [45-47] while injury-induced inflammation in other tissues seems to be more acute in mice than humans [48,49]. Therefore, we evaluated the aged human LEC transcriptome and its acute response to an ex vivo culture model previously developed to investigate the molecular mechanisms of posterior capsular opacification (PCO) [29,50,51].

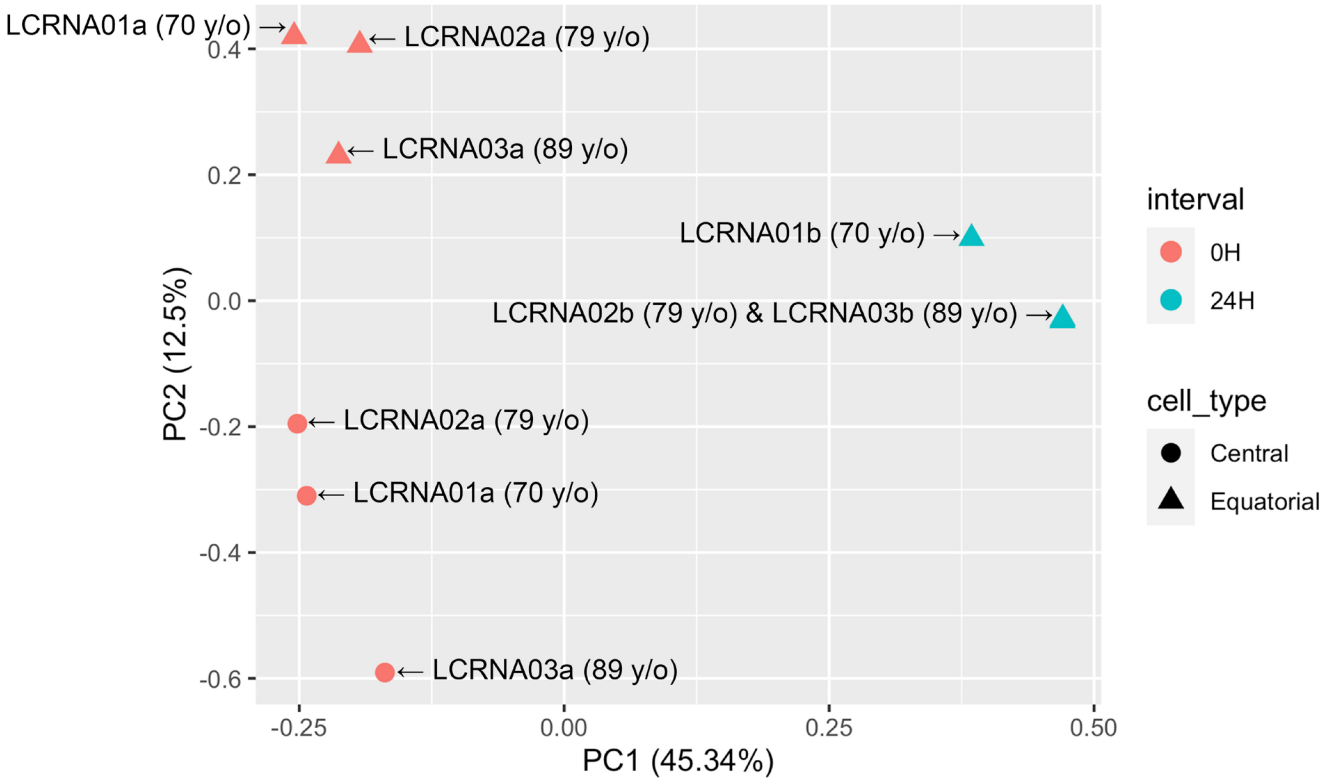

Figure 1. Principal component analysis (PCA) for the human central epithelial cells at 0H and equatorial epithelial cells at 0H and 24H culture. The circles represent the central epithelial cells while the triangles represent the equatorial epithelial cells. The color red represents the 0H culture time point and blue represents the 24H culture time point. Note that two of the 24H equatorial LECs samples (right most triangles) are nearly entirely overlapping. y/o (years old) signifies the age of each donor. Additional details can be found in Appendix 1.

Lenses from human eye bank eyes obtained from a Spanish population (n=3, 70–89 years of age, 2 male, 1 female; see Appendix 1) were subjected to continuous curvilinear capsulorhexis to isolate the central lens epithelium while the equatorial lens epithelium was prepared for culture in the capsular bag model of PCO [29]. RNA was prepared from capsulorhexis specimens and capsular bags containing equatorial epithelial cells isolated either immediately after dissection or after 24 h of culture, then RNAseq performed. Principal component analysis (Figure 1) revealed that all three biologic replicates for each cell population cluster together well despite the fact that these samples vary in sex, postmortem time and cause of death, with the greatest variance detected between equatorial epithelial cells analyzed before and after culture. These data have been deposited in the Gene Expression Omnibus under accession number GSE186716. Comparisons between the freshly isolated central and equatorial lens epithelium transcriptomes are found in Appendix 2. Comparisons between the freshly isolated equatorial lens epithelium transcriptome and the equatorial epithelium following 24 h of culture in the capsular bag model of PCO transcriptomes are found in Appendix 3.

The aged human lens epithelial cell transcriptome: Consistent with other studies [52,53], regional differences were found in the bulk lens epithelial transcriptomes, with central and equatorial human LECs differentially expressing 720 genes by at least twofold with false discovery rate (FDR) corrected p values of ≤0.05. Further filtering for biologic significance revealed 464 biologically significant DEGs (Appendix 2). Functional predictions via Advaita iPathway analysis revealed that the “calcium signaling pathway” (p value 1.165E-6) was the most impacted pathway, while consistent with known regional differences in the adult lens epithelium, the equatorial epithelium expressed numerous genes regulating epithelial cell proliferation (p value 3.600E-4), cell adhesion (p value 4.400E-8), cell junction assembly (p value 8.700E-8), and cation transport (p value 2.500 E-8) at higher levels than the central lens epithelium (Figure 2). As

we also noticed that many DEGs expressed at higher levels in the equatorial epithelium than central epithelium such as Fibrillin 1 (FBN1) which are known components of the ciliary zonule, we compared these DEGs with a list of proteins found in the zonule in a previous study [54] and found that equatorial LECs express genes encoding 12 known zonule components at higher levels than the central LECs, while genes encoding 4 zonule components are expressed at lower levels in the equatorial epithelium (p value of 2.8E-3; Table 1).

Despite these differences, the central and equatorial epithelium share 76% of their most abundantly expressed (top 10% highest expression as measured by FPKM) genes in common (1465 out of 1921, Appendix 4). Many of these shared, highly expressed genes exhibit enriched expression in the lens as assessed by iSyTE (Appendix 2), and are known to be important for lens biology, including crystallins, genes encoding components of the lens capsule, and developmentally important transcription factors (Table 2).

Comparison between the aged mouse and human lens epithelium transcriptome: The mouse is a common animal model to study lens biology and cataract development. Mouse genetics methods are robust and the mouse lens expresses most genes relevant to human cataract etiology. While differences in the molecular composition of mouse and human lenses have been investigated for specific classes of proteins [44-46], a global comparative transcriptomic analysis was lacking until now. Here we compare the transcriptome of 70–89 year old human lens epithelial cells to that of 24 month old (aged) mice, as mice of this age have been proposed to be biologically equivalent to 70 year old humans [55].

Aged human and mouse LECs express similar numbers of genes at sufficient levels to likely affect lens biology. The 10% most abundant mRNAs found in the aged human central epithelium were mapped to their direct mouse gene homologs. The resulting set of 1787 mapped homologs was then compared with the genes expressed in the top 10% of the aged human equatorial lens epithelium as well as the 24 month old mouse lens epithelium. Of the genes exhibiting the highest expression in the human central epithelium, 36% were also ranked in the top 10% of the human equatorial epithelium and aged mouse lens epithelium (637 total genes ranked in the top 10% of expression in all three cell types). An additional 32% of top 10% expressed genes (575 out of 1,787) whose mRNAs were highly abundant in both the central human and aged mouse LECs but not the human equatorial epithelium (See Table 3 for representative examples, entire list is found in Appendix 5). A similar comparison was made between the aged human equatorial LEC transcriptome and aged mouse lens epithelium which revealed that 40% of the highly expressed genes with known human/mouse homologs were among the most expressed in both cell populations (See Table 4 for representative examples, entire list is found in Appendix 6).

These data show that the bulk transcriptome of the aged mouse lens epithelium is generally more similar to equatorial human LECs than central as would be expected since a smaller lens diameter would result in a larger proportion of the lens epithelial cells in the lens epithelium being "equatorial" due to tissue geometry. The aged mouse lens epithelium thus appears to be a good representation of the aged human equatorial lens epithelium which is the cell population that gives rise to Soemmering's ring and visual axis opacification (VAO)/PCO following cataract surgery.

Acute effect of "cataract surgery" on the human lens epithelial cell transcriptome: We have previously shown that mouse lens epithelial cells acutely trigger inflammatory responses following lens fiber cell removal [15,17,56]. Here we evaluate whether human equatorial LECs respond similarly when placed in the human capsular bag model of posterior capsular opacification. As shown in Figure 1, principal component analysis revealed that 24 h of culture caused a major shift in the human equatorial LEC transcriptome compared to that found in freshly isolated equatorial epithelial cells. Further, all three cultured equatorial LEC samples had very similar gene expression profiles despite differences in sex, age, cause of death and postmortem time which indicates that the injury response of human LECs is reproducible. Comparison of the human equatorial LEC transcriptome following 24 h of culture with that from freshly isolated equatorial LECs revealed that 6269 genes were differentially expressed while filtering this list under criteria previously proposed to predict biologically significant DEGs [38] revealed a total of 4042 biologically significant DEGs (Appendix 3).

iPathway analysis of the genes whose expression changes in human equatorial epithelial cells after 24 h of culture revealed the "Cytokine-cytokine receptor interaction" is the pathway most impacted by 24 h of culture (Figure 3A,B; p value=1.120e-8). Consistent with this, many of the genes with the greatest fold change increase in expression play known roles in tissue inflammation or innate immunity (Table 5). Notably, many of the inflammatory cytokine genes upregulated in human LECs after fiber cell removal encode proteins whose concentrations elevate in human aqueous humor within 20 h of cataract surgery (19; Table 6), however other such cytokines either do not change in expression in LECs in the first 24 h of culture, or are not expressed by human LECs at all (Appendix 7).

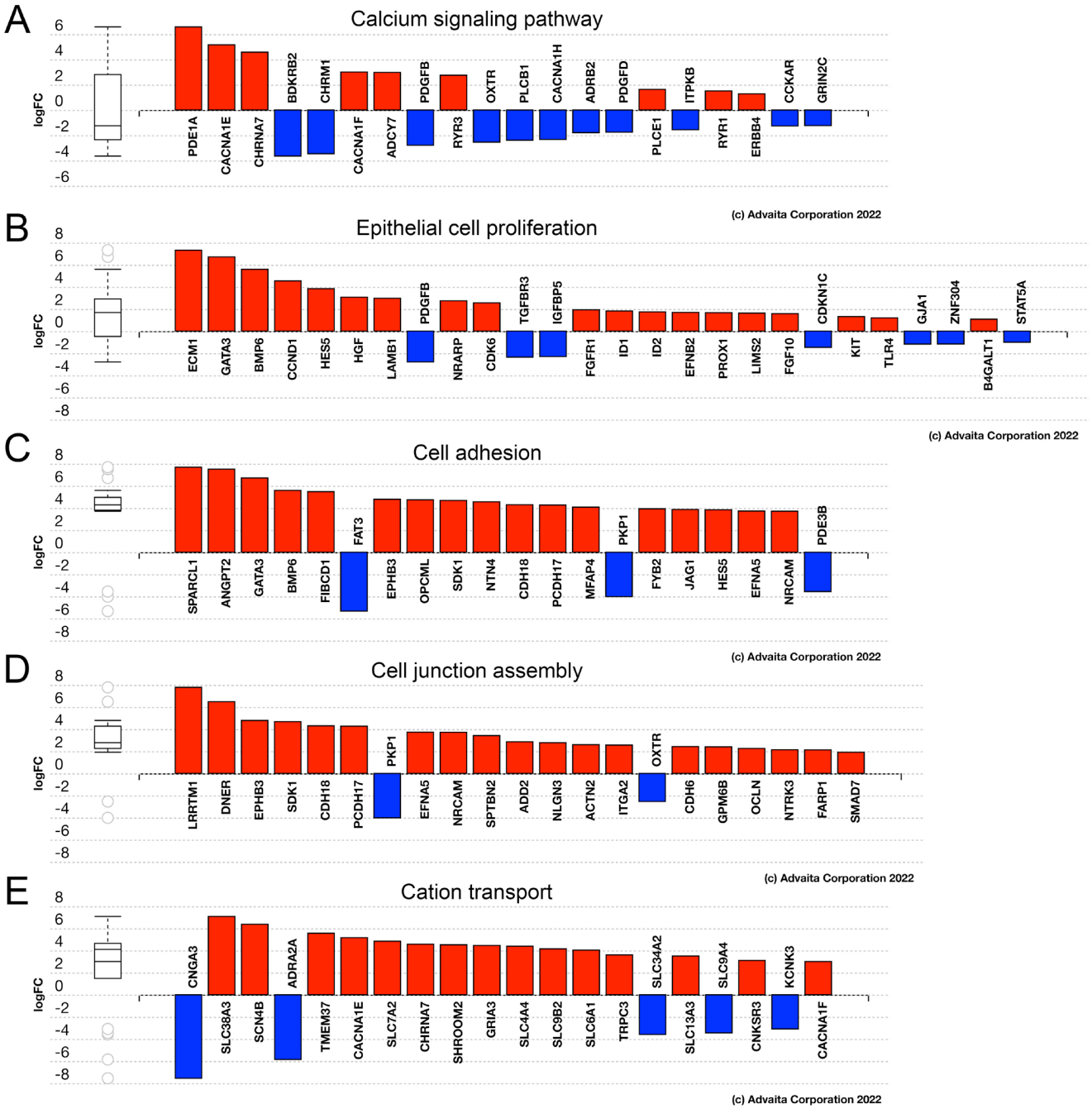

Figure 2. Advaita iPathway analysis of differentially expressed pathways/genes between central lens epithelial compared to equatorial epithelial. **A**: Bar graph showing the "differentially expressed pathway genes on calcium signaling pathway (p-value 1.65E-6) expressed more highly in the 0H human equatorial LECs. **B**: Bar graph showing the "differentially expressed genes annotated to epithelial cell proliferation" (p value 3.600E-4) expressed more highly in the 0H human equatorial LECs. **C**: Bar graph showing the "differentially expressed genes annotated with cell adhesion" (p value 4.400E-18) expressed more highly in the 0H human equatorial LECs. **D**: Bar graph showing the "differentially expressed genes annotated with cell junction assembly" (p value 8.700E-8) expressed more highly in the 0H human equatorial LECs. **E**: Bar graph showing the "differentially expressed genes annotated with cation transport" (p value 2.500E-8) expressed more highly in the 0H human equatorial LECs. Red bars are genes that are expressed more highly in the equatorial lens epithelial cells compared to central lens epithelial cells. Blue bars are genes that are expressed more highly in central lens epithelial cells compared to equatorial lens epithelial cells.

Table 1. Genes encoding known components of the ciliary zonule that are differentially expressed in central versus equatorial lens epithelial cells freshly isolated from human cadaver lenses.

Gene code	Gene name	Central FPKM	Equatorial FPKM	Fold change	FDR
ECM1	Extracellular Matrix Protein 1	1.54	246.85	159.53	1.97E-02
TINAGL1	Tubulointerstitial Nephritis Antigen Like 1	1.95	53.45	27.41	5.70E-06
FRZB	Frizzled Related Protein	0.10	2.18	20.99	3.83E-03
ADAMTSL4	ADAMTS like 4	9.65	123.25	12.77	8.02E-07
FBN1	Fibrillin	1.06	11.38	10.70	4.48E-08
TGM2	Transglutaminase 2	9.65	123.25	12.77	8.02E-07
NID1	Nidogen 1	23.07	106.95	4.64	1.68E-10
EFEMP1	EGF Containing Fibulin Extracellular Matrix Protein 1	44.96	202.07	4.49	1.59E-15
COL18A1	Collagen Type XVIII Alpha 1 Chain	1.07	4.67	4.36	3.10E-04
NID2	Nidogen 2	1.03	4.41	4.29	2.66E-04
MEGF6	Multiple EGF like Domains 6	5.05	18.81	3.72	1.47E-08
LOXL1	Lysyl Oxidase Like 1	47.07	97.95	2.08	1.42E-02
COL4A4	Collagen Type IV Alpha 4 Chain	57.13	27.48	−2.08	2.76E-02
ABI3BP	ABI Family Member 3 Binding Protein	65.00	26.28	−2.47	2.71E-05
TIMP3	TIMP Metallopeptidase Inhibitor 3	1482.04	586.92	−2.53	3.30E-04
SERPINA1	Serpin Family A Member 1	5.60	0.45	−12.28	1.81E-02

FPKM- fragments per kilobase million; FDR- false discovery rate corrected p value.

Table 2. Examples of genes known to be important for lens biology that are highly expressed in both the central and equatorial human lens epithelium freshly isolated from cadaver lenses.

Gene code	Gene name	Central FPKM	Equatorial FPKM	References
CRYAB	Crystallin Alpha B	2929.52	5271.15	[95]
CRYBB2	Crystallin Beta B2	2012.33	8481.76	[96]
VIM	Vimentin	1025.50	800.17	[70]
FTL	Ferritin Light Chain	572.87	550.88	[97]
AQP1	Aquaporin 1	409.85	624.03	[73]
CRYAA	Crystallin Alpha A	332.91	274.72	[98]
GJA1	Gap Junction Protein Alpha 1	270.28	119.79	[99]
CITED2	Cbp/p300 Interacting Transactivator with Glu/Asp rich carboxyl-terminal domain 2	232.53	220.31	[100]
FOXE3	Forkhead box E3	222.00	385.26	[101]
COL4A3	Collagen Type IV Alpha 3 Chain	116.46	63.98	[102]
AQP5	Aquaporin 5	82.39	106.34	[103]
SIX3	SIX homeobox 3	78.04	96.13	[104]
HSPG2	Heparan Sulfate Proteoglycan 2	71.18	92.89	[105]
PAX6	Paired Box 6	44.26	44.03	[67]
ITGB1	Integrin Subunit Beta 1	23.23	29.17	[106,107]

Table 3. Examples of genes highly expressed (top 10%) in both aged human central epithelial cells and 24 month old mouse lens epithelial cells.

Gene code	Gene name	Central human FPKM	Mouse FPKM	References
CRYAB/Cryab	Crystallin Alpha B	3222.44	9608.55	[95]
CRYBB2/Crybb2	Crystallin Beta B2	2205.12	16,545.00	[96]
ALDH1A1/Aldh1a1	Aldehyde Dehydrogenase Family 1, Subfamily A1	1612.38	237.60	[108]
VIM/Vim	Vimentin	1124.82	1271.65	[70,109]
CRIM1/Crim1	Cysteine Rich Transmembrane MBP Regulator 1	543.42	683.85	[108]
CRYGS/Crygs	Crystallin, Gamma S	481.65	2628.69	[110]
AQP1/Aqp1	Aquaporin 1	450.93	80.43	[73]
CCN2/Ccn2	Cellular Communication Network Factor 2	387.64	182.95	[111]
CRYAA/Cryaa	Crystallin Alpha A	362.02	18,815.73	[98]
CITED2/Cited2	Cbp/p300-Interacting Transactivator with Glu/Asp-Rich C-terminal Domain 2	255.61	46.77	[100]
FOXE3/Foxe3	Forkhead Box E3	244.40	264.49	[101]
COL4A3/Col4a3	Collagen Type IV Alpha 3	128.13	165.46	[102]

Other significantly impacted pathways included those participating in “neuroactive ligand-receptor interactions” (Figure 3C,D; p value=6.959e-7) while the “epithelial to mesenchymal transition” pathway was also predicted to be impacted (Figure 3E; p value 0.042). Notably, as would be expected in cells transitioning to a mesenchymal phenotype, several genes known to be important for lens function also downregulate in human equatorial LECs after 24 h of culture (Table 7).

Table 4. Representative genes highly expressed (top 10%) in both aged human equatorial epithelial cells and 24 month old mouse lens epithelial cells.

Gene code	Gene name	Equatorial human FPKM	Mouse FPKM	References
CRYBB2/Crybb2	Crystallin Beta B2	9219.07	16,373.51	[96]
CRYAB/Cryab	Crystallin Alpha B	5739.75	9504.28	[95]
ALDH1A1/Aldh1a1	Aldehyde Dehydrogenase 1 Family Member A1	1464.38	234.90	[108]
VIM/Vim	Vimentin	871.48	1257.50	[70,109]
CRYGS/Crygs	Crystallin Gamma S	863.88	2602.04	[110]
CRIM1/Crim1	Cysteine Rich Transmembrane BMP Regulator 1	560.18	676.14	[112]
FOXE3/Foxe3	Forkhead Box E3	420.07	261.49	[101]
CRYAA/Cryaa	Crystallin Alpha A	300.71	18,617.30	[98]
SPARC/Sparc	Secreted Protein Acidic and Cysteine Rich	193.81	703.63	[113]
BFSP1/Bfsp1	Beaded Filament Structural Protein 1	86.67	662.61	[114]
DKK3/Dkk3	Dickkopf 3	79.58	1128.94	[115]
COL4A2/Col4a2	Collagen Type IV Alpha 2 Chain	44.07	735.50	[102]

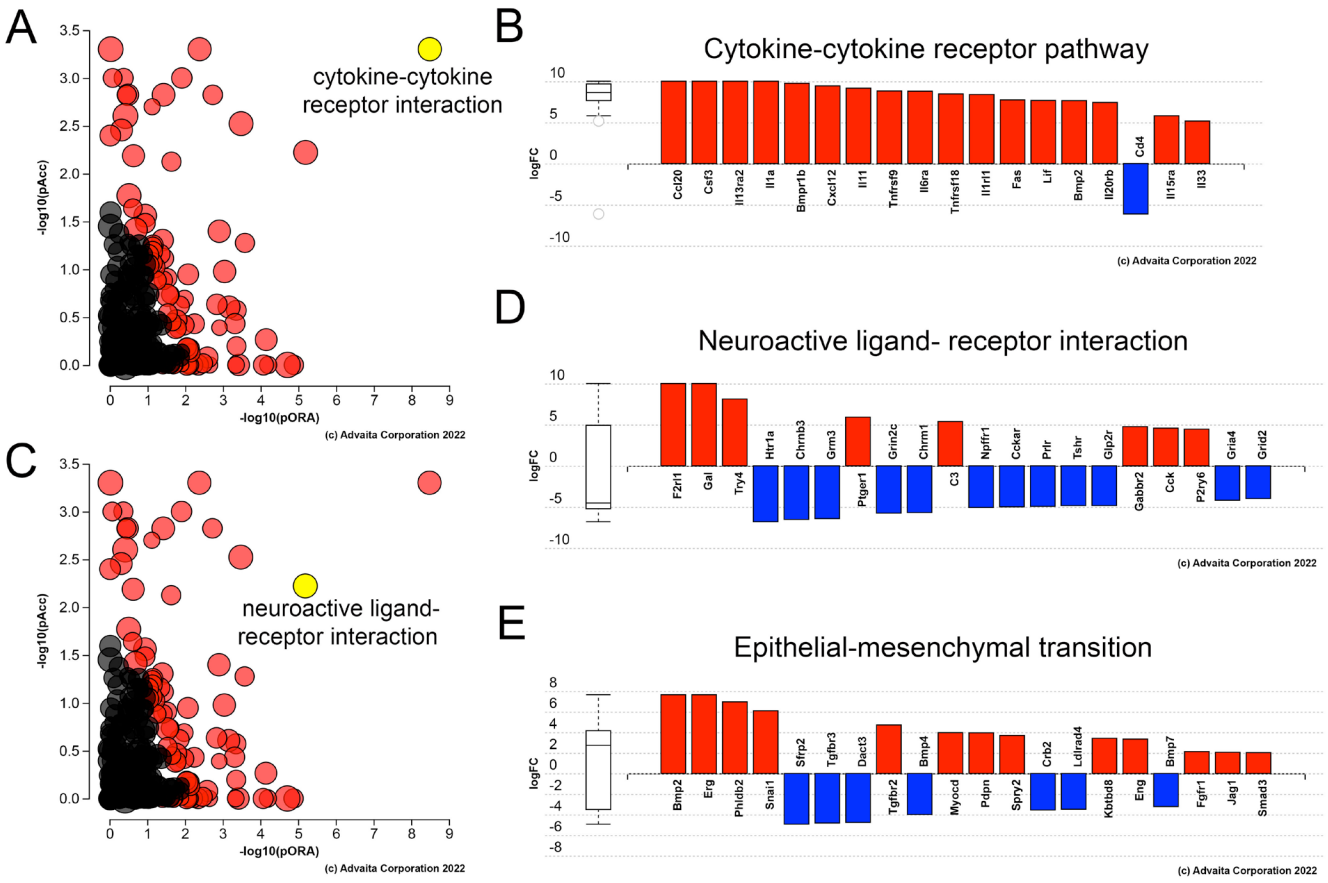

Figure 3. Advaita iPathway analysis of differentially expressed pathways/genes between 0H and 24H human equatorial lens epithelial cells. **A**: Impact analysis of the DEGs suggest that the KEGG pathway map "cytokine-cytokine receptor interaction" (yellow dot) is likely to be the most significantly impacted pathway in the 24H human equatorial LECs. **B**: Bar graph showing the cytokine-cytokine receptor interaction genes that are differentially expressed in the 24H human equatorial LECs. **C**: Impact analysis showing that the second most significant pathway in the 24H human equatorial LECs represent genes involved in the "neuroactive ligand-receptor interaction" pathway (yellow dot). **D**: Bar graph showing the "neuroactive ligand-receptor interaction" pathway genes differentially expressed in the 24H human equatorial LECs. E) Bar graph showing the "epithelial to mesenchymal transition" pathway genes differentially expressed in the 24H human equatorial LECs.

Comparison between the human equatorial epithelium transcriptional response to the lens capsular bag model to the in vivo response of aged mouse lens epithelial cells to lens fiber cell removal: We previously reported that both the aged and young mouse LECs transcriptome is drastically remodeled by 24 h after fiber cell extraction in an in vivo model of cataract surgery [15,17]. Comparison of these DEGs with the list of genes differentially expressed at least twofold in human equatorial epithelial cells following 24 h of culture revealed that human and mouse LECs commonly upregulate 555 homologous genes and downregulate 290 homologous genes (Appendix 8). As the KEGG pathway "cytokine-cytokine receptor interaction" was the most impacted pathway in both human (Figure 3A,B) and mouse LECs [15,17] at 24 h after fiber cell removal, we next assessed the similarities between this response in human and mouse LECs and found that 20 genes in this pathway were upregulated in LECs from both species following injury including various tumor necrosis factor receptors and interleukin receptors (Table 8). However, while the KEGG pathway "neuroactive receptor-ligand interaction was predicted to be an impacted pathway in injured human LECs (Figure 3), this was not seen in injured aged mouse LECs [17] although some genes mapping to this pathway such as F2RL1, GAL, GABBR2, and C3 were upregulated in LECs of both species at 24 hours post lens fiber cell removal.

As the inflammatory response of LECs may contribute to the formation of capsular bag associated myofibroblasts

Table 5. Genes with the largest fold change in expression in aged human equatorial epithelial cells following 24 h of culture. Those with known roles in inflammation and/or innate immunity are in bold font.

Gene code	Gene name	Equatorial FPKM	Equatorial FPKM	Fold change	FDR
MMP1	Matrix Metallopeptidase 1	0.00	94.34	29,959.76	5.10E-14
PTGS2	Prostaglandin-Endoperoxide Synthase 2	0.00	33.92	21,579.19	7.97E-5
CSF3	Colony Stimulating Factor 3	0.00	83.00	21,371.37	2.29E-129
MMP3	Matrix Metallopeptidase 3	0.00	91.09	19,587.73	1.46E-11
IL13RA2	Interleukin 13 Receptor Subunit Alpha 2	0.00	74.80	14,107.77	1.01E-40
CXCL8	C-X-C Motif Chemokine Ligand 8	0.06	576.41	9537.83	4.16E-60
LAMC2	Laminin Subunit Gamma 2	0.06	535.19	8279.12	4.81E-14
MMP10	Matrix Metallopeptidase 10	0.01	104.99	5537.03	9.77E-67
CLMP	CXADR like Membrane Protein	0.00	9.32	5467.92	8.49E-10
FGF5	Fibroblast Growth Factor 5	0.00	7.74	5350.30	1.10E-26
MMP9	Matrix Metallopeptidase 9	0.00	19.05	5161.34	1.80E-4
CDCP1	CUB Domain Containing Protein 1	0.00	6.79	5127.52	3.76E-8
CD93	CD93 Molecule	0.00	6.08	4708.85	6.57E-9
C2CD4A	C2 Calcium Dependent Domain Containing 4A	0.00	10.64	4250.85	5.21E-12
ESM1	Endothelial Cell Specific Molecule 1	0.01	48.83	3984.72	3.25E-14
RCSD1	RCSD Domain Containing 1	0.00	4.08	3863.62	1.09E-9
BNC1	Basonuclin 1	0.00	6.47	3681.52	1.47E-9
RHCG	Rh Family C Glycoprotein	0.00	12.22	3661.28	6.15E-10
CXCL1	C-X-C Motif Chemokine Ligand 1	0.26	857.96	3235.67	3.10E-10
FSRL1	F2R like Trypsin Receptor 1	0.00	9.46	3215.99	7.38E-44

[21] indicative of fibrotic PCO [11], it was expected that the 24H equatorial human LECs differentially express genes implicated in epithelial to mesenchymal transition (EMT; Figure 3E) similar to our prior observation that mouse LECs upregulate genes associated with EMT by 24 h after lens injury [15,17]. Further, direct comparison of the transcriptomic changes triggered in mouse and human LECs by 24 h PCS found that 23 genes of the MSigDB "HALLMARK EMT" gene set were commonly regulated in both species

Table 6. Inflammatory cytokines whose concentration upregulates in aqueous humor by 20 h post cataract surgery [19] whose mRNA levels also change in human equatorial lens epithelial cells after 24 h in culture.

Gene code	Gene name	Equatorial FPKM	Equatorial FPKM	Fold change	FDR
CSF3	Colony Stimulating Factor 3	0.00	83.00	21,371	2.29E-129
CXCL8	C-X-C Motif Chemokine Ligand 8	0.06	576.41	9537	4.16E-60
CXCL1	C-X-C Motif Chemokine Ligand 1	0.26	857.96	3235	3.10E-10
IL1A	Interleukin 1 Alpha	0.01	29.66	1797	2.09E-83
CXCL12	C-X-C Motif Chemokine Ligand 12	0.00	3.82	693	1.42E-22
KITLG	KIT Ligand	0.04	3.34	75	2.80E-13
CCL2	C-C Motif Chemokine Ligand 2	15.02	222.43	14	8.43E-16
CSF1	Colony Stimulating Factor 1	1.87	17.50	97	4.02E-6
HGF	Hepatocyte Growth Factor	4.57	1.61	−3	1.29E-2
CLEC11A	C-Type Lectin Domain Containing 11A	6.59	1.41	−5	5.34E-6

Table 7. Representative lens marker changes in human equatorial LECs after culture.

Gene code	Gene name	Equatorial FPKM	Equatorial FPKM	Fold change	FDR
LGSN	Lengsin	4.6	0.07	−66	7.7E-7
CRYGD	Gamma D crystallin	10.1	0.2	−56	1.7E-6
SFRP2	Secreted frizzled-related protein 2	310	10	−31	2.3E-34
FGFR3	FGF receptor 3	209	13	−16	7.5E-40
FOXE3	Forkhead Box E3	375.02	26	−14	3.4E-21
HSF4	Heat shock Factor 4	4.4	0.4	−10	4.7E-7
FGFR2	FGF receptor 2	36	4	−9	2.9E-31
CITED2	Cbp/p300 Interacting Transactivator with Glu/Asp Rich Carboxyl-Terminal Domain 2	214.44	32.41	−6.6	2.25E-17
COL4A3	Collagen Type IV Alpha 3 Chain	62.30	9.83	−6.34	3.50E-11
AQP5	Aquaporin 5	103.53	19.17	−5.40	1.82E-3
HSPG2	Heparan Sulfate Proteoglycan 2	90.43	26.32	−3.44	4.16E-5
CRYAB	Crystallin Alpha B	5130.63	1501.50	−3.42	2.68E-8
SIX3	SIX Homeobox 3	93.56	28.24	−3.31	3.11E-12
PAX6	Paired Box 6	42.86	16.23	−2.64	2.27E-5
AQP1	Aquaporin 1	607.53	252.96	−2.40	1.11E-2

including the classical lens EMT markers α-smooth muscle actin and tenascin C (Table 9).

DISCUSSION

Mice have long been the most common animal model used to study mammalian lens development and cataract pathogenesis due to the availability of genetic tools that allow functional testing of genes in vivo. Such studies are generally believed to yield conclusions that further our understanding of human lens biology as mouse lenses express most (if not all) genes implicated in human cataractogenesis [57,58]. Further, mouse cataract surgery models have been developed for the study of human posterior capsular opacification (PCO) [42,59–61] which appear to be appropriate animal models as, like humans [50,62], the remnant capsular bags become populated with a mixture of α-smooth muscle actin expressing myofibroblasts and crystallin expressing dysgenic lens fiber cells at extended times following fiber cell removal [16,61,63,64]. However, while the literature reports several instances where mouse and human lenses express different genes [45-47], the global similarities between the mouse and human lens epithelial cell (LEC) transcriptome had not been investigated, and even less was known about how/whether human LECs, like mouse LECs, acutely alter their transcriptome in response to cataract surgery. This study sought to fill these knowledge gaps.

Both central and equatorial human lens epithelial cells express a common set of genes known to be important for lens biology: The most common surgical therapy for cataracts is phacoemulsification, a procedure that removes the central anterior lens capsule with attached lens epithelial cells and lens fiber cells, leaving the remaining lens capsule and attached equatorial LECs behind to anchor an intraocular lens implant [3,4]. Here, bulk RNAseq profiling of the central versus equatorial epithelial cells revealed that these cell populations have very similar transcriptomes which share most of their most abundant transcripts in common including those encoding transcription factors critical for LEC identity such as PITX3 [65], FOXE3 [66] and PAX6 [67], as well as numerous proteins important for LEC/lens function such as CRYAB (αB-crystallin) [68,69], VIM (vimentin) [70], SFRP1 (secreted frizzled related protein 1) [71], FTL (ferritin light chain) [72] and AQP1 (aquaporin 1) [73]. There are, however, key transcriptomic differences between the central and equatorial cell populations in the human lens epithelium. For instance, equatorial epithelial cells express higher levels of genes important for cell cycle progression such as CDK6 [74], while central epithelial cells express higher amounts of mRNAs encoding cell cycle inhibitors such as CDKN1C [75]. These observations are consistent with experimental findings that revealed that adult lens central epithelial cells seldom proliferate while equatorial epithelial cells continue proliferating throughout life leading to a slow increase in

Table 8. Genes that are members of the cytokine-cytokine receptor KEGG pathway that are differentially regulated during the LEC injury response in both mice and humans.

Gene code	Gene name	Human versus	Mouse versus
CXCL12/Cxcl12	C-X-C Motif Chemokine Ligand 12	Up Bio	Up Bio
IL6R/Il6ra	Interleukin 6 Receptor	Up Bio	Up Bio
RELT/Relt	RELT TNF Receptor	Up Bio	Up Bio
IL4R/Il4r	Interleukin 4 Receptor	Up Bio	Up Bio
IL11/Il11	Interleukin 11	Up Bio	Up Bio
CSF3/Csf3	Colony Stimulating Factor 3	Up Bio	Up Bio
CCL2/Ccl2	C-C Motif Chemokine Ligand 2	Up Bio	Up Bio
IL1A/Il1a	Interleukin 1 Alpha	Up Bio	Up Bio
IL1R1/Il1r1	Interleukin 1 Receptor Type 1	Up Bio	Up Bio
TNFRSF10B/Tnfrsf23	TNF Receptor Superfamily Member 10b	Up Bio	Up Bio
TNFRSF10B/Tnfrsf26	TNF Receptor Superfamily Member 10b	Up Bio	Up Bio
LIF/Lif	Leukemia Inhibitory Factor	Up Bio	Up Bio
GDF15/Gdf15	Growth Differentiation Factor 15	Up Bio	Up Bio
IFNAR2/Ifnar2	Interferon Alpha and Beta Receptor Subunit 2	Up Bio	Up Bio
TNFRSF11B/Tnfrsf11b	TNF Receptor Superfamily Member 11b	Up Bio	Up Bio
TNFRSF10D/Nradd	TNF Receptor Superfamily Member 10d	Up Bio	Up Bio
TNFRSF10D/Tnfrsf23	TNF Receptor Superfamily Member 10d	Up Bio	Up Bio
TNFRSF10D/Tnfrsf26	TNF Receptor Superfamily Member 10d	Up Bio	Up Bio
CLCF1/Clcf1	Cardiotrophin like Cytokine Factor 1	Up Bio	Up Bio
CSF1/Csf1	Colony Stimulating Factor 1	Up Bio	Up Bio
LIFR/Lifr	LIF Receptor Subunit Alpha	Down Bio	Down Bio
CNTFR/Cntfr	Ciliary Neutrophic Factor Receptor	Down Bio	Down Bio
IL11RA/Il11ra1	Interleukin 11 Receptor Subunit Alpha	Down Bio	Down Bio

lens size across the lifespan [76]. Further, consistent with the known differentiation of equatorial epithelial cells into lens fibers, equatorial LECs express higher levels of many genes important for lens fiber cell biology than the central epithelium including those participating the Notch and FGF signaling pathways which regulate lens fiber generation [77,78]. Additionally, numerous genes associated with cell adhesion and cell junction assembly are expressed at higher levels in the human equatorial LECs compared to the central LECs which is consistent with prior reports that found that equatorial LECs upregulate genes associated with cell adhesion [79,80] to set the stage for the proper organization of differentiating lens fiber cells.

Notably, equatorial LECs from aged individuals also express appreciable levels of mRNAs that encode extracellular matrix (ECM) proteins that are components of the zonules, acellular filaments that tether the lens to the ciliary muscle, suggesting that the equatorial lens epithelium plays a role in zonule maintenance. Such a role is consistent with the clinical observation that “dead bag syndrome,” the failure to form Soemmering’s ring following cataract surgery, is associated with zonular fragility and dislocation of the IOL/capsular bag complex from its connections to the ciliary body [81,82]. However, it is currently unclear how LECs which are enclosed by the lens capsule can contribute to the maintenance of zonules which are largely intercalated into the outer surface of the lens capsule matrix [83,84].

Aged mouse lens epithelial cells express similar genes as the aged human lens epithelium: Unlike human cataract surgery, a capsulorhexis is typically not performed in the mouse in vivo cataract surgery model due to the small size of the eye. As both the central and equatorial epithelium are retained in the mouse eye following surgery, potential differences between these cell populations could complicate translating mechanistic conclusions from the mouse model to human. To gauge the likely extent of such differences, we compared our prior transcriptomic profiling of the 24 month old mouse lens epithelium with that of the aged human central and equatorial

Table 9. Genes known to participate in the "EMT" response that are commonly regulated in mouse and human LECs at 24 h following fiber cell removal.

Gene code	Gene name	Human versus	Mouse versus
TNFAIP3/Tnfaip3	TNF Alpha Induced Protein 3	Up Bio	Up Bio
TIMP1/Timp1	TIMP Metallopeptidase Inhibitor 1	Up Bio	Up Bio
PTX3/Ptx3	Pentraxin 3	Up Bio	Up Bio
PMEPA1/Pmepa1	Prostate Transmembrane Protein Androgen Induced 1	Up Bio	Up Bio
PLAUR/Plaur	Plasminogen Activator Urokinase Receptor	Up Bio	Up Bio
DPYSL3/Dpysl3	Dihydropyrimidinase like 3	Up Bio	Up Bio
TAGLN/Tagln	Transgelin	Up Bio	Up Bio
TGM2/Tgm2	Transglutaminase 2	Up Bio	Up Bio
THBS1/Thbs1	Thrombospondin 1	Up Bio	Up Bio
NT5E/Nt5e	5′-Nucleotidase Ecto	Up Bio	Up Bio
MMP3/Mmp3	Matrix Metallopeptidase 3	Up Bio	Up Bio
EMP3/Emp3	Epithelial Membrane Protein 3	Up Bio	Up Bio
TPM4/Tpm4	Tropomyosin 4	Up Bio	Up Bio
SERPINE1/Serpine1	Serpin Family E Member 1	Up Bio	Up Bio
ADAM12/Adam12	ADAM Metallopeptidase Domain 12	Up Bio	Up Bio
FLNA/Flna	Filamin A	Up Bio	Up Bio
TNC/Tnc	Tenascin C	Up Bio	Up Bio
GEM/Gem	GTP Binding Protein Overexpressed in Skeletal Muscle	Up Bio	Up Bio
PDLIM4/Pdlim4	PDZ and LIM Domain 4	Up Bio	Up Bio
PLOD3/Plod3	Procollagen-Lysine,2-Oxoglutarate 5-Dioxygenase 3	Up Bio	Up Bio
ACTA2/Acta2	Actin Alpha 2, Smooth Muscle	Up Bio	Up Bio
TNFRSF11B/Tnfrsf11b	TNF Receptor Superfamily Member 11b	Up Bio	Up Bio
CCN1/Ccn1	Cellular Communication Network Factor 1	Up Bio	Up Bio
SLIT3/Slit3	Slit Guidance Ligand 3	Down Bio	Down Bio
PCOLCE/Pcolce	Procollagen C-Endopeptidase Enhancer	Down Bio	Down Bio
FMOD/Fmod	Fibromodulin	Down Bio	Down Bio
ABI3BP/Abi3bp	ABI Family Member 3 Binding Protein	Down Bio	Down Bio
MAGEE1/Magee1	MAGE Family Member E1	Down Bio	Down Bio
SFRP1/Sfrp1	Secreted Frizzled Related Protein 1	Down Bio	Down Bio

Up Bio means upregulated; Down Bio means downregulated and meets biologic significance filtering criteria.

epithelium by identifying genes that have homologs in both mouse and humans. As it was not possible to directly compare transcript abundance between species, we then rank ordered expressed genes based on transcript abundance in each species/cell type then compared which genes contributed to transcripts in the top 10% of abundance. This analysis revealed that approximately 30% of the most abundant genes are shared between all three cell types (human central and equatorial LECs, aged mouse LECs) while another 30%–40% of the most abundant genes are shared between either human central LECs and mouse or human equatorial LECs and mouse, although bulk transcriptome of the aged mouse lens epithelium is more similar to the human equatorial epithelium as would be expected since the human equatorial epithelium makes up 68% of the total lens epithelium [85]. It should be noted that these abundance comparisons likely underestimate the similarities between the mouse and human LEC transcriptome as our decision to compare the 10% most abundantly expressed genes is an arbitrary cutoff. Overall, the similarities in gene expression between the human central and equatorial epithelium and the total mouse lens epithelium suggest that analysis of the response of the entire lens epithelium in the mouse cataract surgery model likely does not greatly bias the results. However, the differences observed do emphasize

the need to validate results obtained in the mouse model with human tissue whenever possible if seeking to relate findings from mouse models to human clinical care.

Aged human lens epithelial cells placed in organ culture for 24 h undergo similar transcriptomic changes as aged mouse lens epithelial cells 24 h post lens injury: Mouse LECs rapidly remodel their transcriptome when separated from their underlying lens fiber cells in both in vivo mouse cataract surgery models [15,17,56] and during explant culture [86], leading these cells to express elevated levels of cytokines/modulators of the innate immune response and genes associated with fibrotic tissue, while downregulating the expression of classical markers of the lens epithelium. While this response appears to be conserved in embryonic chick LECs as well since they upregulate proinflammatory cytokines after just 1 h in explant culture [87], it was unknown whether human LECs left behind following cataract surgery respond similarly.

Here we found that human equatorial lens epithelial cells derived from human cadaver lenses also massively reprogram their transcriptome by 24 h of culture in the lens capsular bag organ culture model of PCO and the global nature of this reprogramming mirrors that seen in the mouse cataract surgery model. Notably, human equatorial LECs were found to downregulate the expression of many LEC preferred genes, while upregulating the expression of fibrotic markers, genes mapping to the KEGG pathway "cytokine-cytokine receptor interactions" as well as other genes known to participate in the innate immune response to tissue injury. Notably, two of the top three most upregulated genes in human equatorial epithelial cells after 24 h in culture, PTGS2/COX2 [88], and CSF3 [89], both participate in tissue inflammation and are also highly upregulated in mouse LECs at both 6 h [56] and 24 h [15,17] after lens fiber cell removal surgery, suggesting that the acute transcriptional response of LECs to lens fiber cell removal is evolutionarily conserved. Further, the cytokines whose levels elevate in human aqueous humor at 20 h following cataract surgery [19] partially overlap with the inflammatory cytokine genes that upregulate in human (and mouse) equatorial LECs after 24 h of culture suggesting that remnant LECs may influence ocular inflammation following cataract surgery by changing the balance of aqueous humor cytokines. However, this overlap is not complete as some cytokines which upregulate in aqueous humor are not expressed in cultured human equatorial epithelial cells. These additional proteins may come from the immune cells which begin to invade the in vivo eye by 18 h PCS [15,90], or other ocular tissues which are influenced by cataract surgery.

Limitations of this study: The present study revealed that human and mouse lens epithelial cells have similar transcriptomes which change similarly in response to lens fiber cell removal such as what is performed during cataract surgery. However, the study design does limit some of the conclusions that can be made.

1) The mouse transcriptomes used for study were derived from inbred mice which were housed in controlled environments, had no apparent pathologies, and lens tissue was isolated immediately after death. In contrast, the human lenses studied were genetically diverse, came from individuals who died natural deaths at a range of ages from diverse causes, with tissue collected at a range of post-mortem times. While the principal component analysis (PCA) revealed that samples obtained from the same cell population isolated from different individuals have quite similar global transcriptomes (Figure 1), it is likely that some genes with bona fide expression differences across cell types were not revealed due to the intrinsic variability of the samples studied and the analysis of samples from only three individuals.

2) The mice and humans used for comparison were "age matched" based on a "frailty" index [55] which assesses a variety of physiologic parameters to set "equivalent ages" between these two species. However, it is recognized that different tissues age at different rates even within an individual [91], and the comparative rates of lens aging between mice and humans have not been directly assessed outside of propensity to develop cataract [92].

3) The human central epithelial samples were preserved in RNAlater immediately after capsulorhexis, but the "uncultured" equatorial epithelial samples were collected approximately 5 - 10 min after hydroexpression of the lens fiber cells. This may have led to an overestimate of the gene expression differences between the central and equatorial epithelium in this study as LECs can trigger gene expression changes as soon as 30 min following a stress [93], which leads to differential expression of approximately 10% of the LEC transcriptome by 6 h PCS [56]. Notably, some genes whose expression rapidly changes in mouse LECs upon lens fiber cell removal, such as the immediate early genes FOS and FOSB which can be upregulated as soon as 30 min after a stress [94], and the inflammatory mediators CXCL14 and CCL2 were detected at lower levels in the central lens epithelium which was immediately preserved versus the equatorial epithelium whose collection was delayed due to its placement within the capsular bag model.

4) The human ex vivo capsular bag model of PCO requires pinning the ciliary body/capsular bag complex into place followed by incubation in cell culture media

supplemented by 5% serum which exposes the equatorial LECs to both the factors present in serum and those that may be produced by the injured ciliary complex, while the injury response of mouse lens epithelial cells was assessed in the eye of a living mouse. These differences likely result in the remnant lens epithelial cells being exposed to a different growth factors/cytokines milieu which could lead to different transcriptomic responses.

Conclusions: This work revealed the baseline similarities and differences between the mouse and human transcriptome and compared their responses to lens fiber cell removal models that mimic modern cataract surgery. Notably, this work confirmed that human LECs, like those in mice, induce the expression of pro-inflammatory cytokines and fibrotic marker genes by 24 h after fiber cell removal, further validating the mouse as a model to study the acute lens injury responses that likely set the stage for the development of posterior capsular opacification, a common negative consequence of modern cataract surgery.

APPENDIX 1. SUPPLEMENTAL TABLE 1.

To access the data, click or select the words "Appendix 1."

APPENDIX 2. SUPPLEMENTAL TABLE 2.

To access the data, click or select the words "Appendix 2."

APPENDIX 3. SUPPLEMENTAL TABLE 3.

To access the data, click or select the words "Appendix 3."

APPENDIX 4. SUPPLEMENTAL TABLE 4.

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APPENDIX 5. SUPPLEMENTAL TABLE 5.

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APPENDIX 6. SUPPLEMENTAL TABLE 6.

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APPENDIX 7. SUPPLEMENTAL TABLE 7.

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APPENDIX 8. SUPPLEMENTAL TABLE 8.

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REFERENCES CITED

1. Wang W, Yan W, Müller A, He M. A Global View on Output and Outcomes of Cataract Surgery With National Indices of Socioeconomic Development. Invest Ophthalmol Vis Sci 2017; 58:3669-76. [PMID: 28728174].
2. Abdulhussein D, Abdul Hussein M. WHO Vision 2020: Have We Done It? Ophthalmic Epidemiol 2023; 30:331-9. [PMID: 36178293].
3. Davis G. The Evolution of Cataract Surgery. Mo Med 2016; 113:58-62. [PMID: 27039493].
4. Liu YC, Wilkins M, Kim T, Malyugin B, Mehta JS. Cataracts. Lancet 2017; 390:600-12. [PMID: 28242111].
5. Lamoureux EL, Fenwick E, Pesudovs K, Tan D. The impact of cataract surgery on quality of life. Curr Opin Ophthalmol 2011; 22:19-27. [PMID: 21088580].
6. Shihan MH, Novo SG, Duncan MK. Cataract surgeon viewpoints on the need for novel preventative anti-inflammatory and anti-posterior capsular opacification therapies. Curr Med Res Opin 2019; 35:1971-81. [PMID: 31328581].
7. Wielders LHP, Schouten J, Nuijts R. Prevention of macular edema after cataract surgery. Curr Opin Ophthalmol 2017; [PMID: 28914687].
8. Morano MJ, Khan MA, Zhang Q, Halfpenny CP, Wisner DM, Sharpe J, Li A, Tomaiuolo M, Haller JA, Hyman L, Ho AC. IRIS Registry Analytic Center Consortium. Incidence and Risk Factors for Retinal Detachment and Retinal Tear after Cataract Surgery: IRIS® Registry (Intelligent Research in Sight) Analysis. Ophthalmol Sci 2023; 3:100314[PMID: 37274012].
9. Clark A, Morlet N, Ng JQ, Preen DB, Semmens JB. Risk for retinal detachment after phacoemulsification: a whole-population study of cataract surgery outcomes. Arch Ophthalmol 2012; 130:882-8. [PMID: 22776926].
10. Awasthi N, Guo S, Wagner BJ. Posterior capsular opacification: a problem reduced but not yet eradicated. Arch Ophthalmol 2009; 127:555-62. [PMID: 19365040].
11. Wormstone IM, Wormstone YM, Smith AJO, Eldred JA. Posterior capsule opacification: What's in the bag? Prog Retin Eye Res 2021; 82:100905[PMID: 32977000].
12. Boswell BA, Korol A, West-Mays JA, Musil LS. Dual function of TGFβ in lens epithelial cell fate: implications for secondary cataract. Mol Biol Cell 2017; 28:907-21. [PMID: 28209733].

13. Meng F, Li J, Yang X, Yuan X, Tang X. Role of Smad3 signaling in the epithelial-mesenchymal transition of the lens epithelium following injury. Int J Mol Med 2018; 42:851-60. [PMID: 29750298].

14. Saika S, Miyamoto T, Ishida I, Shirai K, Ohnishi Y, Ooshima A, McAvoy JW. TGFbeta-Smad signalling in postoperative human lens epithelial cells. Br J Ophthalmol 2002; 86:1428-33. [PMID: 12446380].

15. Jiang J, Shihan MH, Wang Y, Duncan MK. Lens Epithelial Cells Initiate an Inflammatory Response Following Cataract Surgery. Invest Ophthalmol Vis Sci 2018; 59:4986-97. [PMID: 30326070].

16. Shihan MH, Novo SG, Wang Y, Sheppard D, Atakilit A, Arnold TD, Rossi NM, Faranda AP, Duncan MK. αVβ8 integrin targeting to prevent posterior capsular opacification. JCI Insight 2021; 6:e145715[PMID: 34554928].

17. Faranda AP, Shihan MH, Wang Y, Duncan MK. The aging mouse lens transcriptome. Exp Eye Res 2021; 209:108663[PMID: 34119483].

18. Dawes LJ, Duncan G, Wormstone IM. Age-related differences in signaling efficiency of human lens cells underpin differential wound healing response rates following cataract surgery. Invest Ophthalmol Vis Sci 2013; 54:333-42. [PMID: 23211822].

19. Song P, Li P, Geng W, Qin M, Su S, Zhou T, Yuan Y, Zhang G, Wu J, Ji M, Guan H. Cytokines possibly involved in idiopathic epiretinal membrane progression after uncomplicated cataract surgery. Exp Eye Res 2022; 217:108957[PMID: 35077755].

20. De Maria M, Coassin M, Mastrofilippo V, Cimino L, Iannetta D, Fontana L. Persistence of Inflammation After Uncomplicated Cataract Surgery: A 6-Month Laser Flare Photometry Analysis. Adv Ther 2020; 37:3223-33. [PMID: 32440977].

21. Moretti L, Stalfort J, Barker TH, Abebayehu D. The interplay of fibroblasts, the extracellular matrix, and inflammation in scar formation. J Biol Chem 2022; 298:101530[PMID: 34953859].

22. Mack M. Inflammation and fibrosis. Matrix Biol 2018; 68-69:106-21. [PMID: 29196207].

23. Kador PF, Fukui HN, Fukushi S, Jernigan HM Jr, Kinoshita JH. Philly mouse: a new model of hereditary cataract. Exp Eye Res 1980; 30:59-68. [PMID: 7363969].

24. Ehling UH, Favor J, Kratochvilova J, Neuhäuser-Klaus A. Dominant cataract mutations and specific-locus mutations in mice induced by radiation or ethylnitrosourea. Mutat Res 1982; 92:181-92. [PMID: 7088001].

25. Graw J. Cataract mutations as a tool for developmental geneticists. Ophthalmic Res 1996; 28:Suppl 18-18. [PMID: 8727958].

26. Sonntag S, Söhl G, Dobrowolski R, Zhang J, Theis M, Winterhager E, Bukauskas FF, Willecke K. Mouse lens connexin23 (Gje1) does not form functional gap junction channels but causes enhanced ATP release from HeLa cells. Eur J Cell Biol 2009; 88:65-77. [PMID: 18849090].

27. Sundin OH. The mouse's eye and Mfrp: not quite human. Ophthalmic Genet 2005; 26:153-5. [PMID: 16352474].

28. Kuszak JR, Zoltoski RK, Tiedemann CE. Development of lens sutures. Int J Dev Biol 2004; 48:889-902. [PMID: 15558480].

29. D'Antin JC, Barraquer RI, Tresserra F, Michael R. Prevention of posterior capsule opacification through intracapsular hydrogen peroxide or distilled water treatment in human donor tissue. Sci Rep 2018; 8:12739-[PMID: 30143742].

30. Cleary G, Spalton DJ, Zhang JJ, Marshall J. In vitro lens capsule model for investigation of posterior capsule opacification. J Cataract Refract Surg 2010; 36:1249-52. [PMID: 20656145].

31. Yates AD, Achuthan P, Akanni W, Allen J, Allen J, Alvarez-Jarreta J, Amode MR, Armean IM, Azov AG, Bennett R, Bhai J, Billis K, Boddu S, Marugán JC, Cummins C, Davidson C, Dodiya K, Fatima R, Gall A, Giron CG, Gil L, Grego T, Haggerty L, Haskell E, Hourlier T, Izuogu OG, Janacek SH, Juettemann T, Kay M, Lavidas I, Le T, Lemos D, Martinez JG, Maurel T, McDowall M, McMahon A, Mohanan S, Moore B, Nuhn M, Oheh DN, Parker A, Parton A, Patricio M, Sakthivel MP, Abdul Salam AI, Schmitt BM, Schuilenburg H, Sheppard D, Sycheva M, Szuba M, Taylor K, Thormann A, Threadgold G, Vullo A, Walts B, Winterbottom A, Zadissa A, Chakiachvili M, Flint B, Frankish A, Hunt SE, IIsley G, Kostadima M, Langridge N, Loveland JE, Martin FJ, Morales J, Mudge JM, Muffato M, Perry E, Ruffier M, Trevanion SJ, Cunningham F, Howe KL, Zerbino DR, Flicek P. Ensembl 2020. Nucleic Acids Res 2020; 48:D1D682-8. [PMID: 31691826].

32. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat Biotechnol 2019; 37:907-15. [PMID: 31375807].

33. Anders S, Pyl PT, Huber W. HTSeq–a Python framework to work with high-throughput sequencing data. Bioinformatics 2015; 31:166-9. [PMID: 25260700].

34. Chen Y, Lun AT, Smyth GK. From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using Rsubread and the edgeR quasi-likelihood pipeline. F1000Res 2016; 5:1438-version 2; peer review: 5 approved[PMID: 27508061].

35. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol 2010; 11:R25-[PMID: 20196867].

36. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010; 26:139-40. [PMID: 19910308].

37. Storey JD, Tibshirani R. Statistical significance for genome-wide studies. Proc Natl Acad Sci U S A 2003; 100:9440-5. [PMID: 12883005].

38. Manthey AL, Terrell AM, Lachke SA, Polson SW, Duncan MK. Development of novel filtering criteria to analyze RNA-sequencing data obtained from the murine ocular lens

during embryogenesis. Genom Data 2014; 2:369-74. [PMID: 25478318].

39. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 2017; 45:D1D353-61. [PMID: 27899662].

40. Ahsan S, Draghici S. Identifying Significantly Impacted Pathways and Putative Mechanisms with iPathwayGuide. Curr Protoc Bioinformatics 2017; 57:7-.

41. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G. The Gene Ontology Consortium. Gene ontology: tool for the unification of biology. Nat Genet 2000; 25:25-9. [PMID: 10802651].

42. O'Neill LM. Wang. Y, Duncan MK. Modeling Cataract Surgery in Mice. J Vis Exp 2023; •••:202-.

43. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst 2015; 1:417-25. [PMID: 26771021].

44. Faranda AP, Shihan MH, Wang Y, Duncan MK. The effect of sex on the mouse lens transcriptome. Exp Eye Res 2021; 209:108676[PMID: 34146586].

45. Berthoud VM, Minogue PJ, Snabb JI, Dzhashiashvili Y, Novak LA, Zoltoski RK, Popko B, Beyer EC. Connexin23 deletion does not affect lens transparency. Exp Eye Res 2016; 146:283-8. [PMID: 27038752].

46. Ma H, Shih M, Hata I, Fukiage C, Azuma M, Shearer TR. Lp85 calpain is an enzymatically active rodent-specific isozyme of lens Lp82. Curr Eye Res 2000; 20:183-9. [PMID: 10694893].

47. Slingsby C, Wistow GJ, Clark AR. Evolution of crystallins for a role in the vertebrate eye lens. Protein Sci 2013; 22:367-80. .

48. Volk SW, Bohling MW. Comparative wound healing–are the small animal veterinarian's clinical patients an improved translational model for human wound healing research? Wound Repair Regen 2013; 21:372-81. [PMID: 23627643].

49. Zomer HD, Trentin AG. Skin wound healing in humans and mice: Challenges in translational research. J Dermatol Sci 2018; 90:3-12. [PMID: 29289417].

50. Koch CR, D'Antin JC, Tresserra F, Barraquer RI, Michael R. Histological comparison of in vitro and in vivo development of peripheral posterior capsule opacification in human donor tissue. Exp Eye Res 2019; 188:107807[PMID: 31539543].

51. Wormstone IM. The human capsular bag model of posterior capsule opacification. Eye (Lond) 2020; 34:225-31. [PMID: 31745327].

52. Shi Y, De Maria A, Lubura S, Šikić H, Bassnett S. The penny pusher: a cellular model of lens growth. Invest Ophthalmol Vis Sci 2014; 56:799-809. [PMID: 25515574].

53. Bassnett S, Šikić H. The lens growth process. Prog Retin Eye Res 2017; 60:181-200. [PMID: 28411123].

54. De Maria A, Wilmarth PA, David LL, Bassnett S. Proteomic Analysis of the Bovine and Human Ciliary Zonule. Invest Ophthalmol Vis Sci 2017; 58:573-85. [PMID: 28125844].

55. Whitehead JC, Hildebrand BA, Sun M, Rockwood MR, Rose RA, Rockwood K, Howlett SE. A clinical frailty index in aging mice: comparisons with frailty index data in humans. J Gerontol A Biol Sci Med Sci 2014; 69:621-32. [PMID: 24051346].

56. Novo SG, Faranda AP, Shihan MH, Wang Y, Garg A, Duncan MK. The Immediate Early Response of Lens Epithelial Cells to Lens Injury. Cells 2022; 11:3456-[PMID: 36359852].

57. Shiels A, Hejtmancik JF. Mutations and mechanisms in congenital and age-related cataracts. Exp Eye Res 2017; 156:95-102. [PMID: 27334249].

58. Shiels A, Bennett TM, Hejtmancik JF. Cat-Map: putting cataract on the map. Mol Vis 2010; 16:2007-15. [PMID: 21042563].

59. Shirai K, Tanaka SI, Lovicu FJ, Saika S. The murine lens: A model to investigate in vivo epithelial-mesenchymal transition. Dev Dyn 2018; 247:340-5. [PMID: 28480986].

60. Lois N, Taylor J, McKinnon AD, Forrester JV. Posterior capsule opacification in mice. Arch Ophthalmol 2005; 123:71-7. [PMID: 15642815].

61. Call MK, Grogg MW, Del Rio-Tsonis K, Tsonis PA. Lens regeneration in mice: implications in cataracts. Exp Eye Res 2004; 78:297-9. [PMID: 14729361].

62. Hiramatsu N, Nagai N, Kondo M, Imaizumi K, Sasaki H, Yamamoto N. Morphological comparison between three-dimensional structure of immortalized human lens epithelial cells and Soemmering's ring. Med Mol Morphol 2021; 54:216-26. [PMID: 33458799].

63. Manthey AL, Terrell AM, Wang Y, Taube JR, Yallowitz AR, Duncan MK. The Zeb proteins δEF1 and Sip1 may have distinct functions in lens cells following cataract surgery. Invest Ophthalmol Vis Sci 2014; 55:5445-55. [PMID: 25082886].

64. Shihan MH, Kanwar M, Wang Y, Jackson EE, Faranda AP, Duncan MK. Fibronectin has multifunctional roles in posterior capsular opacification (PCO). Matrix Biol 2020; 90:79-108. [PMID: 32173580].

65. Ahmad N, Aslam M, Muenster D, Horsch M, Khan MA, Carlsson P, Beckers J, Graw J. Pitx3 directly regulates Foxe3 during early lens development. Int J Dev Biol 2013; 57:741-51. [PMID: 24307298].

66. Medina-Martinez O, Brownell I, Amaya-Manzanares F, Hu Q, Behringer RR, Jamrich M. Severe defects in proliferation and differentiation of lens cells in Foxe3 null mice. Mol Cell Biol 2005; 25:8854-63. [PMID: 16199865].

67. Cvekl A, Callaerts P. PAX6: 25th anniversary and more to learn. Exp Eye Res 2016; [PMID: 27126352].

68. Andley UP, Song Z, Wawrousek EF, Brady JP, Bassnett S, Fleming TP. Lens epithelial cells derived from alphaB-crystallin knockout mice demonstrate hyperproliferation and genomic instability. FASEB J 2001; 15:221-9. [PMID: 11149910].

69. Brady JP, Wawrousek EF. Targeted disruption of the mouse αB-crystallin gene. Invest Ophthalmol Vis Sci 1997; 38:S935-[PMID: 9023351].

70. Sandilands A, Prescott AR, Carter JM, Hutcheson AM, Quinlan RA, Richards J, FitzGerald PG. Vimentin and CP49/filensin form distinct networks in the lens which are independently modulated during lens fibre cell differentiation. J Cell Sci 1995; 108:1397-406. [PMID: 7615661].

71. Sugiyama Y, Shelley EJ, Wen L, Stump RJ, Shimono A, Lovicu FJ, McAvoy JW. Sfrp1 and Sfrp2 are not involved in Wnt/β-catenin signal silencing during lens induction but are required for maintenance of Wnt/β-catenin signaling in lens epithelial cells. Dev Biol 2013; 384:181-93. [PMID: 24140542].

72. Vanita V, Hejtmancik JF, Hennies HC, Guleria K, Nürnberg P, Singh D, Sperling K, Singh JR. Sutural cataract associated with a mutation in the ferritin light chain gene (FTL) in a family of Indian origin. Mol Vis 2006; 12:93-9. [PMID: 16518306].

73. Schey KL, Gletten RB, O'Neale CVT, Wang Z, Petrova RS, Donaldson PJ. Lens Aquaporins in Health and Disease: Location is Everything! Front Physiol 2022; 13:882550[PMID: 35514349].

74. Tigan AS, Bellutti F, Kollmann K, Tebb G, Sexl V. CDK6-a review of the past and a glimpse into the future: from cell-cycle control to transcriptional regulation. Oncogene 2016; 35:3083-91. [PMID: 26500059].

75. Eggermann T, Binder G, Brioude F, Maher ER, Lapunzina P, Cubellis MV, Bergadá I, Prawitt D, Begemann M. CDKN1C mutations: two sides of the same coin. Trends Mol Med 2014; 20:614-22. [PMID: 25262539].

76. Shui YB, Beebe DC. Age-dependent control of lens growth by hypoxia. Invest Ophthalmol Vis Sci 2008; 49:1023-9. [PMID: 18326726].

77. Zhao H, Yang T, Madakashira BP, Thiels CA, Bechtle CA, Garcia CM, Zhang H, Yu K, Ornitz DM, Beebe DC, Robinson ML. Fibroblast growth factor receptor signaling is essential for lens fiber cell differentiation. Dev Biol 2008; 318:276-88. [PMID: 18455718].

78. Saravanamuthu SS, Gao CY, Zelenka PS. Notch signaling is required for lateral induction of Jagged1 during FGF-induced lens fiber differentiation. Dev Biol 2009; 332:166-76. [PMID: 19481073].

79. Hu Z, Shi W, Riquelme MA, Shi Q, Biswas S, Lo WK, White TW, Gu S, Jiang JX. Connexin 50 Functions as an Adhesive Molecule and Promotes Lens Cell Differentiation. Sci Rep 2017; 7:5298-[PMID: 28706245].

80. Zelenka PS. Regulation of cell adhesion and migration in lens development. Int J Dev Biol 2004; 48:857-65. [PMID: 15558477].

81. Darian-Smith E, Safran SG, Coroneo MT. Zonular and capsular bag disorders: a hypothetical perspective based on recent pathophysiological insights. J Cataract Refract Surg 2023; 49:207-12. [PMID: 36700888].

82. Werner L. The dead bag syndrome. J Cataract Refract Surg 2022; 48:517-8. [PMID: 35703837].

83. Pan Y, Liu Z, Zhang H. Research progress of lens zonules. Adv Ophthalmol Pract Res 2023; 3:80-5. [PMID: 37846380].

84. Bassnett S. Zinn's zonule. Prog Retin Eye Res 2021; 82:100902[PMID: 32980533].

85. Šikić H, Shi Y, Lubura S, Bassnett S. A full lifespan model of vertebrate lens growth. R Soc Open Sci 2017; 4:160695[PMID: 28280571].

86. Upreti A, Padula SL, Tangeman JA, Wagner BD, O'Connell MJ, Jaquish TJ, Palko RK, Mantz CJ, Anand D, Lovicu FJ, Lachke SA, Robinson ML. Lens Epithelial Explants Treated with Vitreous Humor Undergo Alterations in Chromatin Landscape with Concurrent Activation of Genes Associated with Fiber Cell Differentiation and Innate Immune Response. Cells 2023; 12:501-[PMID: 36766843].

87. DeDreu J, Basta MD, Walker JL, Menko AS. Immune Responses Induced at One Hour Post Cataract Surgery Wounding of the Chick Lens. Biomolecules 2023; 13:1615-[PMID: 38002297].

88. Simon LS. Role and regulation of cyclooxygenase-2 during inflammation. Am J Med 1999; 106:5B37S-42S. [PMID: 10390126].

89. Hamilton JA. Colony-stimulating factors in inflammation and autoimmunity. Nat Rev Immunol 2008; 8:533-44. [PMID: 18551128].

90. Way C, Swampillai AJ, Lim KS, Nanavaty MA. Factors influencing aqueous flare after cataract surgery and its evaluation with laser flare photometry. Ther Adv Ophthalmol 2023; 15:25158414231204111[PMID: 38107248].

91. Nie C, Li Y, Li R, Yan Y, Zhang D, Li T, Li Z, Sun Y, Zhen H, Ding J, Wan Z, Gong J, Shi Y, Huang Z, Wu Y, Cai K, Zong Y, Wang Z, Wang R, Jian M, Jin X, Wang J, Yang H, Han JJ, Zhang X, Franceschi C, Kennedy BK, Xu X. Distinct biological ages of organs and systems identified from a multi-omics study. Cell Rep 2022; 38:110459[PMID: 35263580].

92. Wolf NS, Li Y, Pendergrass W, Schmeider C, Turturro A. Normal mouse and rat strains as models for age-related cataract and the effect of caloric restriction on its development. Exp Eye Res 2000; 70:683-92. [PMID: 10870527].

93. Wang X, Garcia CM, Shui YB, Beebe DC. Expression and regulation of alpha-, beta-, and gamma-crystallins in mammalian lens epithelial cells. Invest Ophthalmol Vis Sci 2004; 45:3608-19. [PMID: 15452068].

94. Bahrami S, Drabløs F. Gene regulation in the immediate-early response process. Adv Biol Regul 2016; 62:37-49. [PMID: 27220739].

95. Muranova LK, Strelkov SV, Gusev NB. Effect of cataract-associated mutations in the N-terminal domain of αB-crystallin (HspB5). Exp Eye Res 2020; 197:108091[PMID: 32533979].

96. Zhao WJ, Xu J, Chen XJ, Liu HH, Yao K, Yan YB. Effects of cataract-causing mutations W59C and W151C on βB2-crystallin structure, stability and folding. Int J Biol Macromol 2017; 103:764-70. [PMID: 28528950].

97. Moravikova J, Honzik T, Jadvidzakova E, Zdrahalova K, Kremlikova Pourova R, Korbasova M. Hereditary hyperferritinemia-cataract syndrome in three Czech families: molecular genetic testing and clinical implications. J AAPOS 2020; 24:352-.

98. Jia ZK, Fu CX, Wang AL, Yao K, Chen XJ. Cataract-causing allele in CRYAA (Y118D) proceeds through endoplasmic reticulum stress in mouse model. Zool Res 2021; 42:300-9. [PMID: 33929105].

99. Quan Y, Du Y, Wu C, Gu S, Jiang JX. Connexin hemichannels regulate redox potential via metabolite exchange and protect lens against cellular oxidative damage. Redox Biol 2021; 46:102102[PMID: 34474393].

100. Chen Y, Doughman YQ, Gu S, Jarrell A, Aota S, Cvekl A, Watanabe M, Dunwoodie SL, Johnson RS, van Heyningen V, Kleinjan DA, Beebe DC, Yang YC. Cited2 is required for the proper formation of the hyaloid vasculature and for lens morphogenesis. Development 2008; 135:2939-48. [PMID: 18653562].

101. Brownell I, Dirksen M, Jamrich M. Forkhead Foxe3 maps to the dysgenetic lens locus and is critical in lens development and differentiation. Genesis 2000; 27:81-93. [PMID: 10890982].

102. Kelley PB, Sado Y, Duncan MK. Expression of Collagen IV subtypes in the developing lens capsule. Matrix Biol 2002; 21:415-23. [PMID: 12225806].

103. Petrova RS, Nair N, Bavana N, Chen Y, Schey KL, Donaldson PJ. Modulation of Membrane Trafficking of AQP5 in the Lens in Response to Changes in Zonular Tension Is Mediated by the Mechanosensitive Channel TRPV1. Int J Mol Sci 2023; 24:9080-[PMID: 37240426].

104. Anchan RM, Lachke SA, Gerami-Naini B, Lindsey J, Ng N, Naber C, Nickerson M, Cavallesco R, Rowan S, Eaton JL, Xi Q, Maas RL. Pax6- and Six3-mediated induction of lens cell fate in mouse and human ES cells. PLoS One 2014; 9:e115106[PMID: 25517354].

105. Rossi M, Morita H, Sormunen R, Airenne S, Kreivi M, Wang L, Fukai N, Olsen BR, Tryggvason K, Soininen R. Heparan sulfate chains of perlecan are indispensable in the lens capsule but not in the kidney. EMBO J 2003; 22:236-45. [PMID: 12514129].

106. Pathania M, Wang Y, Simirskii VN, Duncan MK. β1-integrin controls cell fate specification in early lens development. Differentiation 2016; 92:133-47. [PMID: 27596755].

107. Simirskii VN, Wang Y, Duncan MK. Conditional deletion of beta1-integrin from the developing lens leads to loss of the lens epithelial phenotype. Dev Biol 2007; 306:658-68. [PMID: 17493607].

108. Lassen N, Bateman JB, Estey T, Kuszak JR, Nees DW, Piatigorsky J, Duester G, Day BJ, Huang J, Hines LM, Vasiliou V. Multiple and additive functions of ALDH3A1 and ALDH1A1: cataract phenotype and ocular oxidative damage in Aldh3a1(-/-)/Aldh1a1(-/-) knock-out mice. J Biol Chem 2007; 282:25668-76. [PMID: 17567582].

109. Capetanaki Y, Smith S, Heath JP. Overexpression of the vimentin gene in transgenic mice inhibits normal lens cell differentiation. J Cell Biol 1989; 109:1653-64. [PMID: 2793935].

110. He Y, Kang J, Song J. ATP differentially antagonizes the crowding-induced destabilization of human γS-crystallin and its four cataract-causing mutants. Biochem Biophys Res Commun 2020; 533:913-8. [PMID: 33004175].

111. Ma B, Kang Q, Qin L, Cui L, Pei C. TGF-β2 induces transdifferentiation and fibrosis in human lens epithelial cells via regulating gremlin and CTGF. Biochem Biophys Res Commun 2014; 447:689-95. [PMID: 24755068].

112. Zhang Y, Fan J, Ho JW, Hu T, Kneeland SC, Fan X, Xi Q, Sellarole MA, de Vries WN, Lu W, Lachke SA, Lang RA, John SW, Maas RL. Crim1 regulates integrin signaling in murine lens development. Development 2016; 143:356-66. [PMID: 26681494].

113. Weaver MS, Sage EH, Yan Q. Absence of SPARC in lens epithelial cells results in altered adhesion and extracellular matrix production in vitro. J Cell Biochem 2006; 97:423-32. [PMID: 16211577].

114. Song S, Landsbury A, Dahm R, Liu Y, Zhang Q, Quinlan RA. Functions of the intermediate filament cytoskeleton in the eye lens. J Clin Invest 2009; 119:1837-48. [PMID: 19587458].

115. Wang Y, Mahesh P, Wang Y, Novo SG, Shihan MH, Hayward-Piatkovskyi B, Duncan MK. Spatiotemporal dynamics of canonical Wnt signaling during embryonic eye development and posterior capsular opacification (PCO). Exp Eye Res 2018; 175:148-58. [PMID: 29932883].