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Identification of global gene expression differences between human lens epithelial and cortical fiber cells reveals specific genes and their associated pathways important for specialized lens cell functions

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Abstract

Purpose: In order to identify specific genes that may play important roles in maintaining the specialized functions of lens epithelial and fiber cells, we have analyzed the global gene expression profiles of these two cell types in the human lens. This analysis will also reveal those genes that are exclusively expressed in the epithelial and cortical fiber cells and those genes that may play important roles in the differentiation of epithelial cells to mature fiber cells.

Methods: Oligonucleotide microarray hybridization was used to analyze the expression profiles of 22,215 genes between adult (average age greater than 56 years) human lens epithelial and cortical fiber cells. The expression levels of selected genes were further compared by semi-quantitative RT-PCR and selected genes were functionally clustered into common categories using the EASE bioinformatics software package.

Results: Analysis of three separate microarray hybridizations revealed 1,196 transcripts that exhibit increased expression and 1,278 transcripts that exhibit decreased expression at the 2 fold or greater level between lens epithelial cells and cortical fiber cells on all three of the arrays analyzed. Of these, 222 transcripts exhibited increased expression and 135 transcripts exhibited decreased expression by an average of 5 fold or greater levels on all three arrays. Semi-quantitative RT-PCR analysis of 21 randomly selected genes revealed identical expression patterns as those detected by microarray hybridization indicating that the microarray data are accurate. Functional clustering of the identified gene expression patterns using the EASE program revealed a wide variety of biological pathways that exhibited altered expression patterns between the two cell types including mRNA processing, cell adhesion, cell proliferation, translation, protein folding, oxidative phosphorylation, and apoptosis, among others.

Conclusions: These data reveal novel and previously identified gene expression differences between lens epithelial and cortical fiber cells. The gene expression differences indicate distinct pathways and functions important for the specialization of lens epithelial and fiber cells and provide insight into potential mechanisms important for lens cell differentiation.

The eye lens provides an excellent model for studying the process of cellular specialization, proliferation, differentiation, and maturation since it is composed of only one cell type in

different stages of cytodifferentiation and continues to grow throughout life. The anterior surface of the lens is covered by a proliferative, simple, cuboidal epithelium under which lie a core of differentiated and elongated fiber cells. Epithelial cells are induced to exit the cell cycle at the equator of the lens where, during the differentiation process, they elongate and lose their organelles, including the nucleus, mitochondria, endoplasmic reticulum, and golgi apparatus [1,2].

The lens epithelium is essential for the growth, differentiation, and homeostasis of the entire organ [2,3]. The epithelial cells contain the highest levels of enzymes and transport systems in the lens [4-6] and, consistent with a lens maintenance function, are the first cells of the lens to be exposed to environmental insults [4,6] including UV light, smoke, and other agents associated with lens damage. Multiple studies suggest that the lens epithelium is capable of communicating with the underlying fiber cells [7] and direct damage to the lens epithelium and its enzyme systems is known to result in cataract formation [8-11]. In contrast to the lens epithelium, differentiating fiber cells contain large numbers of ribosomes [12,13] necessary for synthesizing cellular components such as the plasma membrane [14], cytoskeleton [15], and the lens crystallins [16] which are essential for cell elongation and differentiation. These fiber cells become packed with crystallin protein, which comprises 90% of the dry weight of the lens [10]. These distinct properties of lens epithelial and cortical fiber cells suggest that these lens components are composed of different proteins and other cellular constituents likely to be contributed by significant differences in gene expression.

The normal polarity of the lens is believed to be related to differences within the eye which could account for maintaining lens cells in two distinct states of cytodifferentiation. The epithelial cells, located on the anterior half of the lens, are bathed in the aqueous humor, while the bow region, the area in which the epithelial cells differentiate into fiber cells, is located at the boundary of the aqueous and vitreous humor. Previous studies have demonstrated that embryonic lenses, which have been surgically rotated so that the epithelial cells face the vitreous humor, result in elongation of the epithelial cells to form structures similar to fiber cells [17,18]. These results suggest that the process of differentiation in the lens may be regulated by the presence of differentiation factors in the aqueous and vitreous humors [17-20]. Some of these factors have more recently been identified to include members of the FGF, IGF, and BMP families [21-29]. Differentiation of lens epithelial cells may also be modulated by molecular cues emanating from surrounding tissues such as the neural retina [30-33] and ciliary body [34,35]. Cell culture studies have demonstrated that fetal lens epithelial cells, co-cultured with fibroblasts derived from the ciliary body, cause epithelial cells to begin to express fiber cell specific genes such as the γ -crystallins [36].

The onset of fiber cell differentiation is known to be accompanied by the upregulation of specific genes including cyclin dependent kinase inhibitor Kip2 (p57) [37] the β - and γ -crystallins [38,39], HSP70 [40], caspase-3 [41], and multiple cyclin dependent kinases [42, 43]. Others have indicated that the ubiquitin dependent pathway is upregulated during lens epithelial cell differentiation [44] and that inhibition of the Src family of kinases is necessary for differentiation of lens cells [45]. These alterations in gene expression reflect the activities of specific transcription factors including elevated levels of c-myc, c-fos, c-jun, c-Maf, Sox-1, and Prox-1 [46-50] that are likely to orchestrate the differential expression of multiple epithelial and fiber specific genes. Recently, *in vitro* and *in vivo* studies have indicated that transcriptional coactivators such as CBP/p300 are also required for lens fiber cell differentiation and for expression of the crystallin genes [51,52].

Although numerous individual growth factors have been implicated in playing a role in the differentiation process of lens epithelial cells, and a hand full of gene expression changes have been identified that correlate with this process, no comprehensive study has examined the

spectrum of gene expression changes that occur during this transformation. Moreover, a multitude of specific gene expression profiles are likely to be important for the specialized functions of lens epithelial and fiber cells. While multiple studies have examined changes in gene expression that occur in many mouse models of cataract, various lens cell lines exposed to agents associated with cataract, or the spectrum of gene expression changes that occur in the presence or absence of a specific gene in the lens, no individual study has examined the gene expression profiles of human lens epithelial and cortical fiber cells. Identification of gene expression differences between lens epithelial cells and cortical lens fiber cells is likely to point to candidate genes involved in the process of differentiation and to those genes responsible for maintaining lens epithelial and fiber cell phenotypes and functions. Despite the difficulty in obtaining sufficient numbers of whole human lenses and in microdissecting pure populations of lens epithelia and cortical fiber cells, it is important that these studies be conducted with actual lenses since no tissue culture model can accurately mimic the unique environment that surrounds the lens or the physiological conditions responsible for orchestrating and maintaining the precise stages of lens cell differentiation in humans.

In the present report, we have utilized oligonucleotide microarrays to compare the global gene expression profiles between matched lens epithelial and cortical fiber cells. We demonstrate that 2,463 genes exhibit altered expression levels of 2 fold or greater between lens epithelial cells relative to cortical fibers. Of these, 222 genes are upregulated and 135 genes are downregulated in the lens epithelium at the level of 5 fold or greater level. Functional clustering of the identified genes revealed multiple biological pathways that are significantly altered between epithelial and fiber cells including mRNA processing, cell adhesion, cell proliferation, translation, protein folding, oxidative phosphorylation, and apoptosis among others. These data shed light on those gene expression changes underlying the specific composition and functions of lens epithelial and fiber cells and provide a basis for those gene expression changes associated with the molecular events that occur during lens cell differentiation.

METHODS

Tissue collection and RNA preparation: Clear whole human lenses were obtained from organ donors within 24 h postmortem from the Lions Eye Bank of Oregon and the West Virginia Eye Bank. Whole lenses were microscopically examined for opacities and any lens exhibiting opacity was discarded from the present study. Clear lenses were microdissected into epithelium and cortical fibers, the cells in the outer most portion of the lens that are currently or have recently undergone the process of differentiation. Nuclear fiber cells, those cells making up the embryonic nucleus and center most portion of the lens were excluded from the present study. Matching lens epithelia and cortical fibers were used for each of three microarray studies. There were twelve lenses used for chip number 1 (average age 56.7 years, ranging from 47-65), 11 lenses for chip number 2 (average age 57.9 years, ranging from 49-66) and 11 lenses for chip number 3 (average age 59.0 years, ranging from 52-69). An additional 12 lenses were used for semi-quantitative RT-PCR confirmation of differentially expressed genes (average age 58.2 years, ranging from 50-67). It is important to note that the average age between all of the groups of lenses used in this study are not statistically different from one another and that matching lens epithelia and cortical fibers were used to eliminate the possibility of individual variability. Total RNA was isolated from these tissues using the Trizol method.

Microarray procedure and analysis: The quality and quantity of RNA obtained from the pooled lens epithelia and corresponding cortical fibers was determined using a Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA) according to the manufacturers protocol. Briefly, a small amount of RNA from each sample was loaded on a microgel, electro-phoresed, scanned and analyzed for the quantity and integrity of the 18S and 28S ribosomal RNA bands to ensure that the same amount of total RNA was examined for both the lens epithelium and fiber samples.

First and second strand cDNAs were synthesized from 2-5 µg of total RNA using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen, Gaithersburg, MD) and the oligo-dT₂₄-T7 primer (5'-GGC CAG TGA ATT GTA ATA CGA CTC ACT ATA GGG AGG CGG-3') according to the manufacturer's instructions. cRNA was synthesized and labeled with biotinylated UTP and CTP by in vitro transcription using the T7 promoter coupled double stranded cDNA as a template and the T7 RNA Transcript Labeling Kit (Enzo Life Sciences Inc., Farmingdale, NY). Briefly, double stranded cDNAs synthesized from the previous steps were washed twice with 70% ethanol and resuspended in 22 µl of RNase free H₂O. The cDNA was incubated with 4 µl each of 10X Reaction Buffer, Biotin Labeled Ribonucleotides, DTT, RNase Inhibitor Mix, and 2 µl of 20X T7 RNA Polymerase for 5 h at 37 °C. The labeled cRNA was separated from unincorporated ribonucleotides by passing through a CHROMA SPIN-100 column (BD Biosciences, San Jose, CA) and precipitated at -20 °C for 1 h to overnight.

The cRNA pellet was resuspended in 10 µl of RNase free H₂O and 10 µg was fragmented by heat and ion mediated hydrolysis at 95 °C for 35 min in 200 µM Tris-acetate, pH 8.1, 500 mM KOAc, and 150 mM MgOAc. The fragmented cRNA was hybridized for 16 h at 45 °C to HG_U133A oligonucleotide arrays (Affymetrix, Santa Clara, CA) containing 22,283 probe sets representing 22,215 gene or extended sequence tag (EST) sequences. Arrays were washed at 25 °C with 6X SSPE (0.9 M NaCl, 60 mM NaH₂PO₄, 6 mM EDTA and 0.01% Tween-20) followed by a stringent wash at 50 °C with 100 mM MES, 0.1 M [Na⁺], and 0.01% Tween-20. The arrays were then stained with phycoerythrin conjugated streptavidin (Molecular Probes, Eugene, OR) and the fluorescence intensities were determined using a laser confocal scanner (Hewlett-Packard, Palo Alto, CA).

The microarray analysis was conducted with GeneSpring 6.1 Software (Silicon Genetics, Redwood City, CA). Briefly, the raw data was normalized first by setting any and all measurements that are less than 0.01 to 0.01 and normalizing the data to the 50th percentile. The gene lists were then filtered to include only those genes marked as "Present" (P) in at least one of the two samples. If a gene was determined to be "Absent" (A) in both the epithelial sample and the cortical fiber sample, the gene was discarded from the present study. Fold change assessments were determined by comparing the epithelial sample to the cortical fiber sample. The resulting gene lists were then compared across all three of the individual experiments conducted and a final gene list was generated that included only those genes detected to be differentially expressed between lens epithelial cells and cortical fibers by an average of at least 2 fold or greater across all 3 of the microarrays. A Student's t-test was used to calculate the p value for the average fold change for each gene in the lens epithelial and cortical fiber cell samples and indicate the likelihood that the calculated fold changes are accurate. Since genes are identified on Affymetrix chips by a probe set identification number and not by gene name, genes that were spotted at multiple loci on the array were treated as if they were different genes. Genes detected to be altered on all three of the array experiments conducted were used for further confirmation and functional clustering. Genes detected to be differentially expressed on two of the three arrays were excluded from this study.

Semi-quantitative RT-PCR confirmations: Twenty one genes were selected for use in semi-quantitative RT-PCR confirmations of the hybridization results. Gene specific primers were designed using the GenBank database (National Center for Biotechnology Information, Bethesda, MD). All primers were designed to cross intron/exon boundaries. The primer sequences, abbreviations, GenBank accession numbers, annealing temperatures, and product lengths for all gene specific primers used in this study are indicated in Table 1. Semi-quantitative RT-PCR was performed using 100 ng of RNA with a commercial RT-PCR system used in accordance with the manufacturer's protocol (One-Step; Invitrogen). Products were separated by gel electrophoresis on 1.5% agarose gels and visualized by ethidium bromide

staining. Product formation for indicated genes was linear over the PCR cycles used. All PCR products were sequenced to ensure product authenticity.

Functional clustering and over representation analysis of differentially expressed genes: Genes identified to be differentially expressed by an average of 2 fold or greater levels were analyzed for significant functional gene clusters using the EASE bioinformatics software package. This software package was used to rank functional clusters by statistical over representation of individual genes in specific categories relative to all genes in the same category on the array. The functional clusters used by EASE were derived from the classification systems of Gene Ontology, Proteome's "At A Glance," SwissProt keywords, and Interpro protein domains.

RESULTS

2,463 gene expression differences were detected between lens epithelial and cortical fiber cells.: Analysis of gene expression differences between matched human lens epithelial cells and cortical fiber cells was conducted using Affymetrix HG_U133A microarrays as described. Comparison of the gene expression data for 22,215 genes represented by 22,283 separate probe sets (each probe set containing 10 perfect match and 10 one base pair mismatch probe sequences) between human lens epithelial cells and cortical fiber cells identified 2,463 genes whose transcript levels differed by 2 fold or greater levels on all three chips examined. There were 747 genes detected to be expressed (present) in lens epithelial cells but absent in lens cortical fiber cells while 422 genes were detected to be expressed in cortical fiber cells (present) but absent in epithelial cells (Figure 1). An additional 1,294 genes were detected to be expressed (present) at different levels in both cells types (Figure 1). There were 1,196 genes identified to be increased and 1,278 genes decreased in lens epithelia relative to cortical fibers by an average of 2 fold or greater levels on all three of the arrays analyzed (Figure 2). Of the transcripts identified to be increased in epithelia relative to cortical fibers, 222 were increased by an average of 5 fold or greater while 135 of the transcripts identified to be decreased in epithelia relative to cortical fibers were decreased at the 5 fold or greater level. All of the raw Affymetrix microarray data for the 3 lens epithelial RNA samples and the 3 lens cortical fiber RNA samples are available at the Gene Expression Omnibus (GEO) website under the accession number GSE2256.

Confirmation of gene expression differences by semi-quantitative RT-PCR: In order to confirm the accuracy of the microarray data, semi-quantitative RT-PCR was conducted on candidate and randomly selected genes that were detected to exhibit altered expression levels on all 3 of the arrays analyzed. RNA was isolated from an additional 12 lens epithelial and matched cortical fiber cells (average age 58.2 years) and 100 ng of RNA was used for the RT-PCR confirmations. Eight transcripts exhibiting increased expression and eight transcripts exhibiting decreased expression at the 2 fold or greater level between lens epithelial cells and cortical fiber cells were selected for confirmation. These included aquaporin 1, chloride channel 2, pax 6, inhibitor of metalloproteinase 3, gap junction protein alpha 1, insulin like growth factor binding protein 5, an EST, and a hypothetical protein which all exhibited increased expression in epithelia relative to cortical fibers according to the microarray data and metallothionein IG, metallothionein IH, glutathione synthetase, lens epithelial protein, peroxiredoxin 6, nuclear receptor coactivator 2, laminin receptor 1, and a hypothetical protein which all exhibited decreased expression in lens epithelia relative to cortical fibers according to the microarray data. Five additional genes, protocadherin gamma subfamily C, a hypothetical protein, an unknown protein, an EST, and transcription elongation factor A, which exhibited identical transcript levels between lens epithelial and cortical fiber cells on all three chips, were used as controls.

All 21 of these genes exhibited the same trends in gene expression as detected by microarray analysis (Figure 3) using RNA isolated from an addition 12 lenses. The levels of all 5 of the control genes selected were nearly identical between lens epithelia and cortical fiber cells while the other 16 genes examined exhibited significant differences in expression levels between the two RNA samples, confirming the results detected by microarray analysis and providing confidence in the data.

Functional clustering of differentially expressed transcripts: All genes exhibiting increased or decreased expression between lens epithelial and cortical fiber cells by an average of 2 fold or greater levels on all three of the arrays examined were analyzed for significant enrichment with respect to various categories of gene function using the EASE bioinformatics package. Selected categories enriched within the mRNAs increased or decreased at the 2 fold or greater level, with an EASE score of less than 0.05, are depicted in Figure 4 and Figure 5. Biological processes such as mRNA processing, caspase activation, glycolysis, cell fate commitment, cell proliferation, antigen presentation, and cell adhesion were detected to be significantly increased in lens epithelial cells relative to cortical fibers (Figure 4). Biological processes such as RNA metabolism, translation, protein folding, ribosome biogenesis, ATP biosynthesis, oxidative phosphorylation, protein disulfide reduction, microtubule based process, and apoptosis were detected to be significantly decreased in lens epithelial cells relative to cortical fibers. The EASE data set, indicating individual genes comprising the selected functional categories depicted in Figure 4 and Figure 5, are posted on the GEO website under the accession number GSE2256. The entire EASE data set listing all of the functional clustering data for those genes upregulated in lens epithelial cells and downregulated in lens epithelial cells is represented in Appendix 1.

DISCUSSION

In the present study, we have compared the relative expression levels of 22,215 genes and ESTs between matched lens epithelium (6-8 mm²) and outer cortical fibers, and confirmed the accuracy of the data by semi-quantitative RT-PCR, and have clustered the differentially expressed genes into functional categories. Microarray analysis of three separately prepared populations of RNA has identified over 2,400 genes that differ in expression between lens epithelial and cortical fiber cells by an average of 2 fold or greater levels. The present gene expression differences are reflective of the specific lens regions examined and further subfractionation is impractical given the availability of human lenses and is beyond the scope of the present report. Of these genes, 222 were detected to be increased and 135 were detected to be decreased at the 5 fold or greater level in lens epithelial cells relative to cortical fiber cells. Semi-quantitative RT-PCR analysis of 21 genes, 8 of which exhibited increased expression, 8 of which exhibited decreased expression, and 5 of which exhibited identical expression levels between lens epithelial and cortical fiber cells, indicate that the gene expression differences detected by microarray analysis are authentic. Functional clustering of genes exhibiting an average expression difference of 2 fold or greater revealed significant alterations in biological processes involved in mRNA processing, caspase activation, glycolysis, cell fate commitment, cell proliferation, antigen presentation, cell adhesion RNA metabolism, translation, protein folding, ribosome biogenesis, ATP biosynthesis, oxidative phosphorylation, protein disulfide reduction, microtubule based process, and apoptosis among others.

Nearly 1,200 genes were identified to be more highly expressed in lens epithelial cells relative to cortical fibers. A number of these genes function in cellular proliferation including members of the tetraspan superfamily of proteins which have been implicated in the regulation of cell growth and differentiation [53], epithelial membrane proteins 1 and 3 and the AXL receptor tyrosine kinase, which has previously been demonstrated to be increased in cataractous mouse

lenses [54] and plays an important role in the control of lens epithelial cell growth and survival [55]. Other genes involved in cell proliferation include vascular endothelial growth factor, insulin like growth factor binding protein 6, glypican 4, cyclin G2, cyclin I, cyclin L1, and tumor necrosis factors 13 and 15. Increased expression of genes involved in cellular proliferation in lens epithelial cells relative to cortical fibers likely reflects cell division in the peripheral germinative zone of the lens epithelium as these cells begin to divide and undergo the process of differentiation.

Another category of genes exhibiting increased expression in lens epithelial cells relative to cortical fibers consists of genes involved in mRNA processing such as heterogeneous nuclear ribonucleoprotein A1, a protein known to bind to the 5' leader sequence of the fibroblast growth factor-2 mRNA to control alternative initiation of translation [56]. This protein is likely to interact with other mRNAs in a similar fashion to control the initiation of translation at multiple sites. A nuclear poly(A) binding protein, which binds to growing poly(A) tails to permit the polyadenylation of RNAs by poly(A) polymerase [57], was also detected to be increased in lens epithelial cells. Multiple splicing factors, some of which are implicated in premRNA processing and others that are likely to play roles in alternative splicing also exhibited increased expression in epithelial cells relative to cortical fibers. Other genes in this category include a couple of pre-mRNA processing factors, a polypyrimidine tract binding protein, and a ribonuclease. The increased expression of genes involved in mRNA processing in epithelial cells relative to cortical fiber cells indicates that the majority of transcription that occurs in the lens is confined to the lens epithelium. This is not surprising since this layer of cells contain active nuclei while most fiber cells have lost their nuclei during the differentiation process.

An additional group of genes involved in the glycolytic pathway was also detected to exhibit overall increased expression in epithelial cells relative to cortical fiber cells. Individual genes include alpha enolase 1, a gene that encodes the alpha enolase enzyme and a structural lens protein, τ -crystallin [58]. Glyceraldehyde-3-phosphate dehydrogenase, a gene primarily involved in an energy yielding step of carbohydrate metabolism and widely used as a housekeeping gene, was also detected to exhibit increased expression in epithelial cells. Multiple phosphofructokinase genes, glucose phosphate isomerase, a lactate dehydrogenase, and a pyruvate kinase gene were also detected to be increased in lens epithelial cells relative to cortical fibers. This group of genes reflects the energy yielding pathway used in the anaerobic environment of the lens and indicates that the majority of ATP that is produced in the lens, and that is necessary for both lens epithelial and fiber cell function, is manufactured in the epithelial cell layer.

Nearly 1,300 genes were detected to be more highly expressed in cortical fiber cells relative to lens epithelial cells. Of the genes detected to exhibit higher levels of expression in cortical fibers, a number of them are involved in translation including multiple tRNA synthetase enzymes responsible for catalyzing the aminoacylation of specific tRNAs by their cognate amino acid. Indeed, previous studies have demonstrated that the onset of lens cell differentiation is accompanied by the enrichment of certain species of tRNA [59]. Numerous translation initiation factors were also detected to be more highly expressed in lens fibers relative to epithelial cells. Increased expression of these factors is necessary for the increased synthesis of cellular components such as the plasma membrane [14], cytoskeleton [15], and the lens crystallins [16] which are all necessary for cell elongation and differentiation. Additional genes include small heat shock protein 27, interleukin 12A, lysophospholipase II, and spectrin. The high abundance of genes involved in translation in lens fiber cells relative to epithelial cells likely reflects the large production of crystallin proteins during lens cell differentiation.

In conjunction with increased expression of genes involved in translation, significant increases were also detected for genes involved in ribosome biogenesis. These include a couple of DEAD

box proteins that are implicated in ribosome and spliceosome assembly [60], opa interacting protein 2, and secernin 3. Many ribosomal protein subunits were also detected to be increased in cortical fiber cells relative to the lens epithelium. In fact, it is well documented that newly differentiating fiber cells contain large numbers of ribosomes relative to lens epithelial cells [12,13] in order to produce components of the plasma membrane and cytoskeleton, and the large amounts of crystallin proteins that are necessary for lens fiber cell differentiation [14-16].

It is well accepted that differentiating lens cells undergo a cellular process similar to apoptosis during which they lose their nuclei and all other organelles. Not surprisingly, our analysis has revealed significant increases in expression of genes involved in the process of cell death in cortical lens fiber cells. Specific examples include bcl-2 associated proteins, members of a family of proteins involved in apoptosis [61], which have previously been implicated in playing an important role in lens fiber cell differentiation [62]. Other genes include a Tax1 binding protein, a gene thought to play a role in inhibition of apoptosis by interacting with the anti-apoptotic zinc finger protein A20 [63], TIA1, and a cytotoxic granule associated RNA binding protein that is believed to function in the Fas mediated apoptotic pathway [64-66]. Other genes include, death associated protein 3, TGF β 1, and growth arrest and DNA damage inducible alpha. The increased expression of genes involved in the apoptotic process in cortical fiber cells relative to epithelial cells is likely to reflect the pseudoapoptotic events that are known to occur during the early stages of lens cell differentiation.

Surprisingly, the majority of crystallin genes were detected to be expressed at some level in the lens epithelium. Of all of the crystallin genes included on the U133A microarrays, α A-, α B-, β A3-, and β B2-crystallins did not exhibit significant differences in expression between the lens epithelium and cortical fibers. β A1-, β A2-, β A4-, β B1-, β B3-, γ A-, γ B-, γ C-, and γ D-crystallins were determined to be expressed at different levels between the two cell types with all being more highly expressed in the cortical fibers. Other genes of interest include filensin and phakinin, members of the lens specific intermediate filament, known as the beaded filament [67], lens major intrinsic protein (MIP), also known as MIP26 and aquaporin 0, which is a polypeptide found exclusively in lens fiber cell membranes that functions as a water channel [68-70], plays a role in gap junction formation [71,72], acts as a cell adhesion molecule [73, 74] and is necessary for γ -crystallin organization [75] and lens epithelial protein, a gene believed to be exclusively expressed in the lens epithelium, were all determined to be more highly expressed in the cortical fiber cells relative to the lens epithelium on all three arrays examined. All of these genes are known to be preferentially expressed in lens fibers cells with the exception of lens epithelial protein, and their increased expression levels in cortical fibers in the present report provide confidence in the accuracy of the data.

Although we cannot rule out the possibility that fiber cell contamination, resulting from the dissection process, may influence the magnitude of the detected gene expression levels, we are confident that the overall trends in gene expression are accurate since these results were obtained with three separate microarrays, representing three separately prepared populations of microdissected lenses that were exhaustively washed and microscopically examined to be free of fiber cells in the epithelial preparations. It is also possible that the gene expression data presented here are reflective of an aging human lens since the lenses used were from older individuals and therefore may not exactly reflect the gene expression profiles of a younger lens. More importantly, these data provide insight into the general gene expression profiles of human central lens epithelial and outer cortical fiber cells. The results provide a data base of genes, and their functional pathways, that differ between these lens regions. The data provide insight into the specialized functions of these cells and those events surrounding their differentiation. The lens provides an excellent model for these types of studies since it allows

for the study of how individual human cells differentiate and specialize *in vivo*. The results of these studies should be useful towards our understanding of more complex systems.

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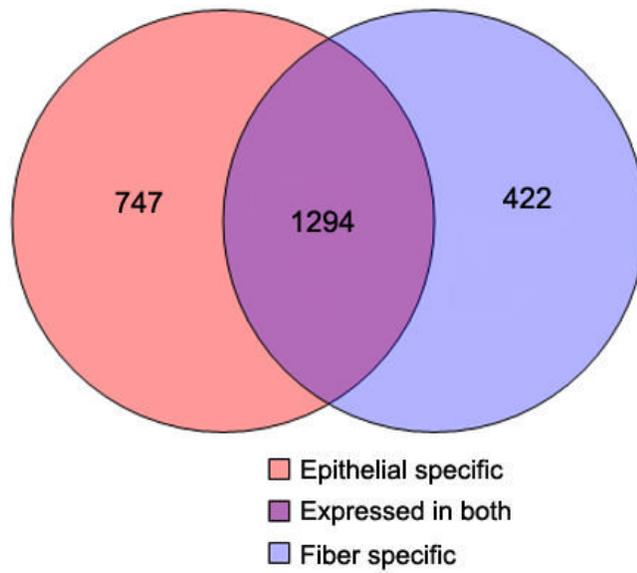


Figure 1. Venn diagram of detected gene expression differences. Venn diagram indicating number of genes expressed only in lens epithelial cells, only in cortical fiber cells, or in both cell types, as detected by microarray analysis.

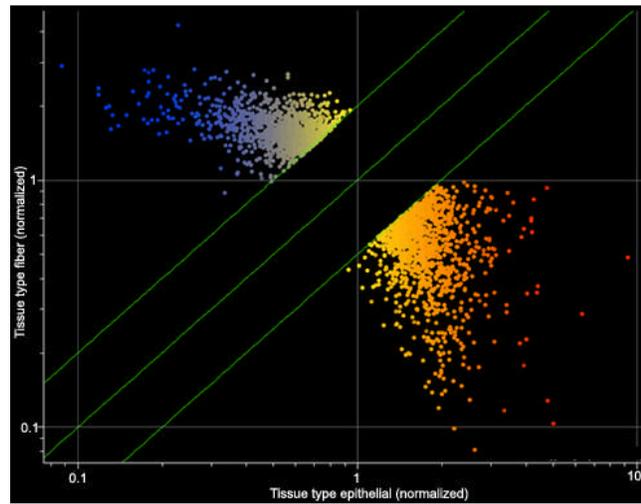


Figure 2.

GeneSpring scatter plot. Each point represents an individual gene that is either upregulated in lens epithelial cells relative to cortical fiber cells (lower right quadrant) or that is downregulated in lens epithelial cells relative to cortical fiber cells (upper left quadrant). The center green line represents no difference in expression level for a given gene between the two cell types while the upper and lower green line indicate the 2 fold change cut off used in the present study.



Figure 3. RT-PCR confirmation of gene expression differences. The expression levels of indicated genes were confirmed by semi-quantitative RT-PCR using 100 ng of RNA isolated from lens epithelial (E) or cortical fiber (F) cells.

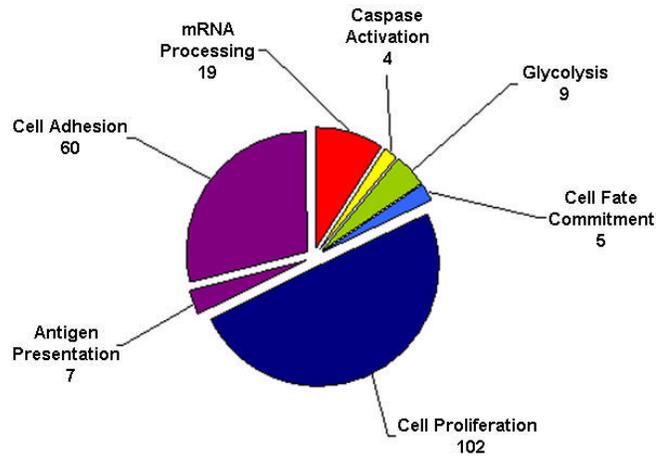


Figure 4. Cluster analysis of genes exhibiting increased expression in lens epithelial cells. Statistical cluster analysis using the program EASE was used to subcategorize genes with significantly increased expression in lens epithelium (compared to cortical fiber cells). The pie chart is a visual enumeration of selected (not all) functional categories. The total number of genes comprising each functional category are indicated. The size of the pie slices represent the relative number of genes with increased expression in the selected categories. The proportions (and the respective counts) of genes in the functional category have no relevance or biological importance; they are simply presented as a broad overview of the EASE analysis. The complete list of the functional categories identified by the EASE analysis is available in Appendix 1. Not all of the genes analyzed fall into one of the functional categories presented in this figure. Also, some genes are represented in more than one functional category. Therefore the total number of genes in the figure do not match the total number of genes with increased expression at the 2 fold or greater level.

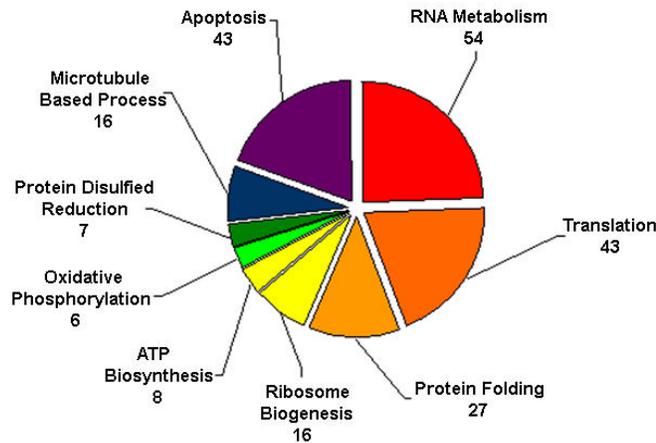


Figure 5.

Cluster analysis of genes exhibiting decreased expression in lens epithelial cells. Statistical cluster analysis using the program EASE was used to subcategorize genes with significantly decreased expression in lens epithelium (compared to cortical fiber cells). The pie chart is a visual enumeration of selected (not all) functional categories. The total number of genes comprising each functional category are indicated. The size of the pie slices represent the relative number of genes with decreased expression in the selected categories. The proportions (and the respective counts) of genes in the functional category have no relevance or biological importance; they are simply presented as a broad overview of the EASE analysis. The complete list of the functional categories identified by the EASE analysis is available in Appendix 1. Not all of the genes analyzed fall into one of the functional categories presented in this figure. Also, some genes are represented in more than one functional category. Therefore the total number of genes in the figure do not match the total number of genes with decreased expression at the 2 fold or greater level.

Table 1

PRIMERS USED FOR RT-PCR

Gene (Abbreviation)	Primer sequence (5'–3')	Annealing temperature (°C)	Product length (bp)	Accession number
Aquaporin 1 (AQP1)	AGCCTCTTATGGGGGTGTTTCTAT	56	417	NM_000385
	ATTCCAAGGTCAGGCAGTAAGTCC	56	417	NM_000385
Chloride channel (CLCA2)	AGCCTGGGCACTGGACTTACA	56	388	NM_006536
	GGGGTGCTTATGCTGGGAGAG	56	388	NM_006536
EST	GTGAAGGCATCCCTGGTAGA	60	321	BF513244
	TGGCTGAGCTGGTCCTTAAT	60	321	BF513244
	AAGCCACATATGCCTCCAAG	60	336	AI189753
	CATGAAAACGTGGCAAACG	60	336	AI189753
Gap junction protein alpha 1 (GJA1)	AGGCGGAAGCACCATCTCTA	56	372	NM_000165
	CCACCCATCTACCCCATACACC	56	372	NM_000165
Glutathione synthetase (GSS)	ATTTGACCAGCGTGCCATAGAGA	56	372	NM_000178
	AGTGAGTAGAGGCCAGCAAAGGTG	56	372	NM_000178
Hypothetical protein	CGTAGCCTCATTCCCAGGTA	60	375	AL136727
	TGTTTGCTCCTGAGGCTTTT	60	375	AL136727
	GGCCGTATCAAGGAGATTCA	60	349	NM_018104
	CCCTTTGACGTAGGAAACCA	60	349	NM_018104
	TTGATGTCAACATGGGCTGT	60	372	NM_017803
	TGGCTTGTGCAAAGTCTCT	60	372	NM_017803
Insulin-like growth factor binding protein 5 (IGFBP5)	TCTGCTCACCCAGAGACCTT	60	368	AW007532
	GAAGGGGCATGATCAGAAAAA	60	368	AW007532
Laminin receptor 1 (LR1)	CGCTCCAGTCTTCAGTAGGG	60	383	AW304232
	CTACCCTACCATTGCGCTGT	60	383	AW304232
Lens epithelial protein (LENEP)	TCCTCCTCTGCTGCTGGTGTATCA	61	426	NM_018655
	GGTTTCTTAGGGGGCAGTCCATTC	61	426	NM_018655
Metallothionein Ig (MTIg)	GCCTCTTCCCTTCTCGCTTG	56	217	NM_005950
	GACATCAGGCGCAGCAGCTG	56	217	NM_005950
Metallothionein Ih (MTIh)	GAACTCCAGTCTCACCTCGG	56	213	NM_005951
	GACATCAGGCACAGCAGCTG	56	213	NM_005951
Nuclear receptor coactivator 2 (NRC2)	TCAATGCAGGTGAACAAAAGTG	60	342	AI040324
	TTCTACATAGCTGTCCGATG	60	342	AI040324
PAX 6 (PAX6)	CAGGGCAATCGGTGGTAGTAAA	56	520	NM_000280
	TTCTCGGGCAAACACATCTGGA	56	520	NM_000280
Peroxiredoxin 6 (PRDX6)	TCCAACCATCCCTGAAGAAGAA	56	386	NM_004905
	AGCCCTGCAGAGATCCAACAAG	56	386	NM_004905
Protocadherin gamma subfamily c (PCDHGC3)	TTGGTTTGGATCTCGGTAGC	60	368	NM_002588
	TCGGCTCAGCTCATAGGTTT	60	368	NM_002588
Tissue inhibitor of metalloproteinase 3 (TIMP3)	GGGCTGTGCAACTTCGTGGAGA	61	333	NM_000362
	CAGCGGGAAGGGAGGGAAGTGA	61	333	NM_000362
Transcription elongation factor A (TFIIS)	GCGGTTGAAGTGTAGGGAGA	60	340	X57198
	AGTCTTGGCCATCTGATGCT	60	340	X57198
Unknown protein	GGTGACTTCACACGCCATAA	60	302	BC001224
	TCGAGTTGTCCACAGTCAGC	60	302	BC001224

The table lists the sequences, GenBank accession numbers, annealing temperatures, product lengths, and abbreviations for all gene specific primers used in this study.