Proteome Analysis of Lens Epithelia, Fibers, and the HLE B-3 Cell Line

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Purpose. The purpose of this study is to compare the protein composition of the B-3 line of transformed human lens epithelial (HLE) cells to that of freshly dissected HLE cells. This provides baseline data on lens cell proteins from fresh lens cells and from the B-3 cell line, which is often used as a model system for the lens.

Methods. Human lens epithelial cells adherent to the lens capsule were dissected into central (undifferentiated) and peripheral (partially differentiated) populations. Fully differentiated human lens fiber cells were isolated from the outer cortical layers of the lens. HLE B-3 cells were analyzed at several passage levels. Extracts were prepared from each cell type and the proteins resolved by two-dimensional polyacrylamide gel electrophoresis (2-DE). Representative gel patterns were visually compared, spots excised, and trypsin digests prepared. The peptide compositions of the digests were analyzed using either liquid chromatography electrospray ionization tandem mass spectrometry or atmospheric pressure–matrix-assisted laser desorption ionization mass spectrometry, using a liquid chromatography classic ion trap (LCQ) mass spectrometer.

Results. Two-DE patterns were obtained for fresh and cultured cell types. Similar patterns were observed between central and peripheral HLE cells, both of which contained high levels of αA-, αB-, and βB2-crystallins; α-enolase; and aldehyde dehydrogenase. HLE B-3 cultured cells were characterized by a marked loss of crystallins and a relatively higher level of noncrystallin proteins—most notably, high molecular weight, acidic proteins. Whereas subunit d of adenosine triphosphate (ATP) synthase, αB-crystallin, galectin, glyceraldehyde-3-phosphate dehydrogenase, α-enolase, actin, peptidylprolyl isomerase A, phosphatidylethanolamine-binding protein, and vimentin were present in both fresh and cultured lens epithelium, only the high abundance of α-enolase, galectin-1, and vimentin suggested that B-3 cells were lens derived.

Conclusions. Freshly dissected noncultured HLE cells from both central and peripheral regions contain a high concentration of crystallins that mask the detection of less abundant proteins by 2-DE. Transformation and culture of HLE cells causes a loss of these crystallins and an increase in the relative concentration of other proteins. However, most of these noncrystallin proteins were different from those observed in noncultured HLE cells. These results suggest that transformation markedly alters the protein expression pattern in immortalized HLE cells and that caution should be exercised when using them to study properties of HLE cells in vivo. (*Invest Ophthalmol Vis Sci.* 2003;44:4829–4836) DOI:10.1167/iovs.03-0556

Continuous cell culture has a number of advantages over in vivo tissues. It affords improved reproducibility, ease of application of quantitative techniques, and controlled experimental conditions. Cell lines are widely available and commonly used in the study of a broad variety of cell types (http://www.atcc.org/ provided in the public domain by the American Tissue Type Culture Collection, Manassas, VA).

The ocular lens is an important research subject, in the search for treatment for prevalent eye disease and cataract and as a model for the study of development, differentiation, and aging. Cell lines were derived from lens epithelial cells to assist in these studies. Examples include lines derived from lenses of mouse (αTN4,[1,2] NKR-11[3]), rabbit (N/N1003A[4]), and human (SRA 01–04[5], HLE B-3[6]).

To use cell culture most effectively, it is also necessary to define the limitations of the technique. One of these is the well-known change in gene expression in response to the altered genetics and/or environment of the cell. For example, a recent 2-D gel comparison of membrane proteins from freshly isolated and overnight cultured hepatocytes revealed rapid protein composition changes as early as 5 hours after culturing.[7] Fresh bladder tumors, however, showed strikingly similar expression profiles when compared with their primary cultures.[8] Even in these cultures, there were significant reproducible differences in the levels of several specific proteins. T-cell acute lymphoblastic leukemia cells closely resembled the cells of patients at the time of diagnosis, in surface markers, karyotype, and gene rearrangements, but these cells were not analyzed by two-dimensional (2-D) gels.[9]

Reverse transcription–polymerase chain reaction assays were performed on three lens cell lines (αTN4, N/N1003A, NKR-11) and the results compared with freshly isolated tissue.[10] Several transcripts expressed preferentially in fresh lens cells (βB2-crystallin, γ-crystallin, MIP, MP20, filensin, and CP49) were undetectable in the cell lines. A control gene for eye development, *Pax6*, was present in all three cell lines.

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αA-crystallin transcripts were present in αTN4 cells, but not in N/N1003A or NKR-11 cells.[10] In another study,[11] nuclear matrix proteins of six mammalian lens epithelial cell continuous cultures were compared by 2-D gel electrophoresis (2-DE). Each cell line had a distinct pattern of protein spots, but they shared a similar spot pattern in the acidic pH/45-kDa region that cross-reacted with anti-vimentin antibody. However, no comparison with uncultured cells was made.[11] The immortalized human epithelial cell line, SRA 01/04,[5] transformed by SV40 T antigen, expressed very low levels of αA- and βB2-crystallins compared with primary cultures. Aldose reductase was also detected at low levels, but these were similar to levels in primary cell cultures.[5]

In this work, we analyzed an extensively studied lens continuous culture, HLE B-3 cells, and compared them with native lens cells. The HLE B-3 cells were derived from an infant human lens epithelial culture by AD12-SV40 hybrid virus infection.[6] After 11 passages, the cells no longer produce the characteristic lens protein, αA-crystallin, but they express αB- and βB-crystallins to passage 26.[12] This absence of αA-crystallin in HLE B-3 cells was useful, because it provided a null background for the addition of cloned αA-crystallin.[14] We compared these cells with freshly isolated, noncultured lens epithelial cells and differentiated lens fiber cells using 2-DE and mass spectrometry to identify proteins. These results confirmed that transformation and culture markedly altered the protein composition of HLE B-3 cells and for the first time identified the major proteins found in freshly isolated lens epithelium.

Materials and Methods

Lens Cells

HLE B-3 cells grown at 37°C in a 95% air, 5% CO_2 atmosphere were passaged weekly in Eagle's minimum essential medium (Sigma-Aldrich, St. Louis, MO) containing 20% fetal bovine serum (Gemini Bioproducts, Calabasas, CA), L-glutamine (Gemini Bioproducts), and gentamicin (Sigma-Aldrich). Cells between passages 11 and 14 (22.2–29.4 population-doubling level [PDL]), and 20 and 25 (61.6–86.6, PDL) were rinsed twice in phosphate-buffered saline (PBS), scraped and aspirated, and collected by centrifuging at low speed. Human donor eyes of age 60 to 70 years were obtained from the National Disease Research Interchange (NDRI, Philadelphia, PA) and the North Carolina Eye Bank, a Vision Share (Apex, NC) member eye bank through the Department of Ophthalmology, New Jersey Medical School. The donor inclusion criteria were: no history of chemotherapy, radiation to the head, or recent ventilation; up to 6 hours between death and enucleation; and up to 50 hours between death and storage (isolated tissue frozen at −80°C). This research adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board (IRB) of the New Jersey Medical School. Lenses were decapsulated under a microscope by a posterior approach and the capsule-adherent epithelial cells dissected into central and peripheral regions. Capsule epithelial cell samples were pooled (30–32 lenses) to obtain sufficient protein (≥400 μg/gel) for replicate 2-DE and mass spectrometry analysis. Combined cortical and peripheral nuclear fiber samples were obtained after decapsulation by carefully peeling off the outer 40% by weight of the lens. Protein was extracted by suspending HLE B-3 cells or dissected fresh lens cell pellets in a lysis buffer that contained 8 M urea, 4% 3-([3-cholamidopropyl]dimethylammonio-2-hydroxy-1-propanesulfonate (CHAPS), and 40 mM Tris base. Cells were homogenized with a Teflon pestle designed to fit in a 1.5-mL microcentrifuge tube (Eppendorf, Fremont, CA). The pestle, fixed to an electric motor, rotating at approximately 1000 rpm, was applied to the cells held on ice, for five 30-second intervals. Cells were further disrupted on ice by five 30-second pulses of sonication. Supernatant fractions were obtained after centrifugation at 40,000*g* and then stored at −20°C. Protein was quantified with a bicinchoninic acid assay (Sigma-Aldrich).

Gel Electrophoresis

For Western blots, one-dimensional SDS-polyacrylamide gel electrophoresis was performed, essentially according to the method of Laemmli,[14] with 12% separating gels, 3% stacking gels, and β-mercaptoethanol in the sample. Molecular weight markers were low-range prestained SDS-PAGE standards (Bio-Rad Laboratories, Hercules, CA; see Fig. 3). Proteins were visualized in the gel by staining with Coomassie brilliant blue R-250. For Western blot analysis, proteins were electroblotted from polyacrylamide gels to polyvinylidene difluoride (PVDF) membranes (Sequi-Blot; Bio-Rad Laboratories). Nonspecific binding was blocked with 5% bovine serum albumin. Rabbit anti-γ-crystallin polyclonal antibody was a generous gift from S.-C. Joseph Fu (Department of Biochemistry & Molecular Biology, UMDNJ, NJ Medical School, Newark, NJ). Antibody binding was visualized with a chemiluminescence kit (Perkin Elmer Life Sciences, Boston, MA), according to the manufacturer's instructions.

The first-dimension separation of 2-D gel electrophoresis was performed with a commercial system (IPGphor; Amersham Biosciences, Piscataway, NJ). Isoelectric focusing (IEF) was performed in the first dimension with purchased 13-cm nonlinear pH 3.5 to 10 immobilized pH gradient (IPG) strips (Amersham Biosciences), with a programed voltage gradient. IPG strips were rehydrated in the first-dimension buffer (8 M urea, 2% CHAPS, 0.5% IPG buffer, 1% dithiothreitol [DTT], and a trace amount of bromophenol blue), which also contained the sample (400–480 μg), and IEF was performed using a step gradient up to 8000 V for 10 to 12 hours to give a total of 40,000 V-hours. After equilibration of each strip in 10 mL of SDS-containing buffer (50 mM Tris-Cl [pH 8.8], 6 M urea, 30% vol/vol glycerol, 2% SDS, 1% DTT, and trace bromophenol blue), the second dimension was performed on a 12% polyacrylamide gel, with the first-dimension IPG strip embedded in 0.5% agarose at the top. Proteins were visualized on the gels by silver staining (Silver Plus One kit; Amersham Biosciences) omitting glutaraldehyde to improve recovery of digested proteins.[15]

Protein Identification

Proteins resolved on 2-D gels and silver stained were excised and cut into approximate 1-mm^2 pieces and dried. Gel pieces were destained for 15 minutes in 100 μL of a freshly prepared 1:1 mixture of 100 mM sodium thiosulfate:30 mM potassium ferricyanide and washed in 100 mM ammonium bicarbonate buffer (AmBic) three times for 15 minutes each. Gel samples were again dried and 40 μL of 10 ng/μL trypsin solution in AmBic added to rehydrate gel pieces (Sequencing Grade Modified Trypsin; Promega, Madison, WI). After 15 minutes, excess solution was removed, 60 μL of AmBic was added to cover gel pieces, and samples were incubated at 37°C for 16 hours (overnight). Peptides were then collected by removing the supernatant and washing and sonicating the remaining gel pieces first in 1:1 AmBic-acetonitrile, then in a 1:1 mixture of 5% formic acid with water-acetonitrile. The combined supernatant-wash solutions were then dried, dissolved in 10 μL of 5% formic acid and the digests analyzed either by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESIMS) or atmospheric pressure–matrix assisted laser desorption ionization mass spectrometry (AP-MALDI) using a liquid chromatography classic ion trap (LCQ) mass spectrometer (ThermoFinnigan, San Jose, CA).

Analysis by LC-ESIMS was performed using a 10-cm × 75-μm capillary column (15 μm tip; PicoFrit, New Objectives, Woburn, MA), packed with 5-μm SB-C18 material (Zorbax; Agilent Technologies, Wilmington, DE). Samples were applied to the column through a capillary trapping cartridge (Michrom Bioresources Inc, Auburn, CA), and the peptides separated with a 200-nL/min flow rate mobile phase containing 0.2% acetic acid, 0.005% heptafluorobutyric acid, and 60 minutes of 0% to 30% acetonitrile gradient. Data-dependent tandem mass spectra on major peptide ions were automatically collected with a dynamic exclusion feature to extend analysis to less abundant peptides.

Analysis by AP-MALDI was performed by purifying gel digests using 10-μL disposable pipette tips filled at the tip with a single layer of C18

membrane material (Empore; 3M Bioanalytical, St. Paul, MN). After binding of peptides by drawing the 10-μL digest through the membrane material five times, the sample was washed with 10 μL of 0.1% trifluoroacetic acid, and peptides directly eluted onto a MALDI plate in 1 μL of 10 mg/mL α-cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile, 0.1% trifluoroacetic acid. Spectra were then collected using an AP-MALDI source (Mass Technologies, Burtonsville, MD) and LCQ ion trap. Fifty microscans were collected in MS mode and used to perform data-dependent tandem MS (MS/MS) on the 10 most abundant ions from each sample.

Proteins present in each digested spot were then identified (Sequest software; ThermoFinnigan) to correlate experimental MS/MS spectra with theoretical MS/MS spectra calculated from peptide sequences in a nonredundant database of human proteins (National Center for Biotechnology Information, Bethesda, MD). Confirmation of the proteins identified by computer analysis (Sequest; ThermoFinnigan) from data collected during LC-ESIMS was made if there were at least three MS/MS spectra of doubly charged ions with cross-correlation scores (Xcorr) of 2.0 or greater, or two MS/MS spectra of doubly charged ions with Xcorr scores of 2.0 or greater plus one or more MS/MS spectra of triply charged ions with Xcorr scores of 3.5 or greater that matched a single protein entry. Confirmation of proteins identified by AP-MALDI was made if at least 3 of 10 MS/MS spectra of singly charged ions had Xcorr scores of 1.0 or greater and matched a single protein entry.

Results

Proteins from cultured B-3 cells derived from human lens epithelium were compared with proteins from freshly isolated capsule epithelium and underlying cortical fibers of 60- to 70-year-old human donors. Figure 1 shows the unlabeled 2-D gels of B-3 cells, fresh central epithelium, fresh peripheral epithelium, and cortex peripheral nucleus. Figure 2 is identical, except proteins excised for analysis by MS are circled. The identities of the numbered protein spots are listed in Table 1.

The most striking difference between the freshly isolated tissues and cultured cells was the absence of crystallins in the cultured cells. βB2-, αA-, and αB-crystallins were by far the predominant proteins in both freshly isolated epithelium and cortical fibers (Figs. 1B–D). The only crystallin detected in the cultured cells was αB-crystallin (Fig. 2A, spot 12), and its concentration was markedly less than in noncultured epithelium. The only other proteins found in B-3 cells that were also present in fresh tissue were subunit d of ATP synthase, galectin-1, glyceraldhyde-3-phosphate dehydrogenase (GAPDH), α-enolase (also known as τ-crystallin), actin, peptidylprolyl isomerase A, phosphatidylethanolamine binding protein, and vimentin. GAPDH was not identified in the gels of fresh epithelium shown in Figures 1 and 2. However, based on analysis of additional gels of fresh epithelium that were analyzed by MS but not shown, proteins found in the gels of fresh epithelium shown in Figures 1 and 2 were probably GAPDH. A search and visual inspection of the SWISS-2D-PAGE database indicated that all 9 of the proteins identified in both HLE B-3 cells and fresh epithelium were also found in non-lenticular mammalian cells (http://us.expasy.org/ch2d/, provided in the public domain by the Swiss Institute of Bioinformatics, Geneva, Switzerland). Because of their relatively greater concentration in HLE B-3 cells, compared with other cell types in the database, only the presence of α-enolase, galectin-1, and vimentin suggested that HLE B-3 cells were of lens origin. The presence of αB-crystallin in HLE B-3 cells was not a specific lens marker, because this protein is also highly expressed in other tissues.[16,17] Although most of the proteins found in HLE B-3 cells were not observed in fresh epithelium (Table 1), this result does not indicate that they are absent. Twenty-four of the 43 identified proteins in HLE B-3 cells were represented at least once in a combined library of 4200 normalized and non-normalized expressed sequence tags from human lens (http://neibank.nei.nih.gov/index.shtml/ NEIBANK provided in the public domain by the National Eye Institute, Bethesda, MD).[18]

Because of the great abundance of crystallins in the fresh epithelium, the 2-DE pattern was far less complex than in the HLE B-3 cells. There was a high concentration of αA-, αB-, βB2-crystallins, α-enolase, actin, and aldehyde dehydrogenase (Figs. 1B, 1C, 2B, 2C). To determine whether the high concentration of crystallins was due to contamination of epithelium with fibers during dissection, SDS-PAGE and Western blotting were used to assess the abundance of γ-crystallin, a marker for fiber cell differentiation,[19] in protein isolated from cultured HLE B-3 cells, freshly dissected epithelium, and cortical fibers (Fig. 3). Only cortical fibers contained detectible γ-crystallin (Fig. 3, lane 4). This suggested that the dissected epithelium was largely free of fiber contamination. Further evidence that there was no significant fiber cell contamination was provided by the difference in crystallin composition between 2-DE gels of epithelium (Figs. 1B, 1C, 2B, 2C) and cortical fibers (Figs. 1D, 2D). Cortical fibers contained βA3-, βA4-, and degraded βB1-crystallins,[20] which were absent in dissected epithelium. The absence of γC- and γD-crystallin in the 2-DE gels of cortical fibers was probably caused by the absence of alkylation before the 2-D gel was run. This causes γ-crystallins to oxidize and resolve poorly on 2-DE gels (David LL, unpublished results, 2000).

Central and peripheral epithelium were dissected and analyzed individually by 2-DE. The compositions of the two regions were remarkably similar (compare Figs. 1B and 1C). Proteins involved in differentiation and elongation would be expected in higher abundance in the peripheral epithelium than in the central epithelium. This suggests that proteins initiating differentiation in epithelium are relatively minor components. These results are similar to those of Russell,[21] who found similar 2-D patterns for both central and peripheral human lens epithelium, as well as a high abundance of crystallins.

Discussion

This study provides the most detailed analysis to date of proteins contained in both freshly isolated and cultured HLE cells. The results indicate that, similar to lens fibers, HLE cells contain an abundance of αA-, αB-, and βB2-crystallins. Epithelial cell crystallins may have functions beyond providing a high index of refraction. Epithelial cells derived from αA knockout mice divide more slowly and are less resistant to stress.[13] Recent evidence suggests that the slower growth in the absence of αA may be due to the need for the presence of αA to stabilize microtubules during mitosis.[22] Both lens epithelium and fibers may need the chaperone activity of α-crystallins for proper function.[23] βB2-crystallin may preferentially accumulate in lens epithelium, because it is more resistant than other β-crystallins to posttranslational modifications.[24] Its presence in nonlenticular tissues, as well as lens epithelium, suggests that it has important unknown nonrefractive functions.

α-Enolase and aldehyde reductase were also found in high abundance in lens epithelium. In vertebrate lenses, ranging from lamprey to birds, α-enolase is a major taxon-specific crystallin that is preferentially expressed in lens epithelial cells compared with fiber cells.[25–28] Its function in human lens epithelium is unknown. However, it was recently shown that an alternate translation of the α-enolase m-RNA using an alternate ATG start codon 400 nucleotides downstream of the normal α-enolase start codon produces a 37-kDa protein called

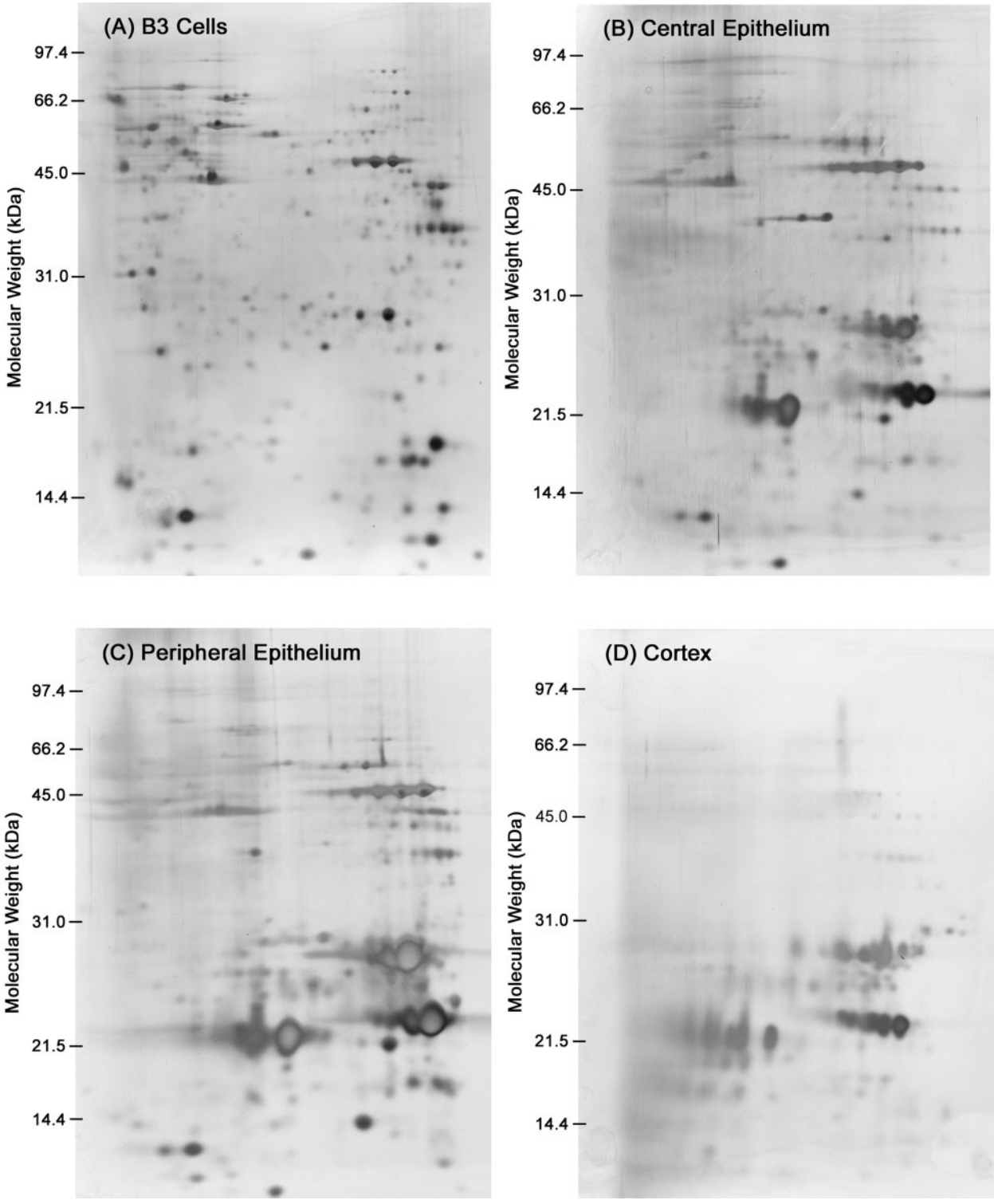

FIGURE 1. Representative 2-DE gels of total proteins from (**A**) cultured HLE B-3 cells, freshly dissected central (**B**) or peripheral (**C**) epithelium, and cortical fiber region (**D**) from human lens. Each silver-stained gel contained approximately 400 μg of protein. pH increases from *left* to *right* in each gel.

myc-binding protein-1 (MYC-1).[29] MYC-1 functions to regulate c-*myc* promoter activity. Because c-*myc* may play a role in lens epithelial cell differentiation,[30] the presence of MYC-1 is of interest. Because of their molecular weight of approximately 40 kDa, the smaller forms of α-enolase in spots marked 21 in the central epithelium (Fig. 2B) were first thought to be MYC-1. However, these proteins contained peptides from the N-terminal region of α-enolase, and therefore we conclude they resulted from C-terminal degradation of the protein instead of an alternate start codon. Nevertheless, because of the abundance of α-enolase in lens epithelium, the possible role of MYC-1 in lens warrants closer examination.

High levels (1%–2% of soluble protein) of aldehyde dehydrogenase 1 (ALDH1) have been reported in the human lens.[31]

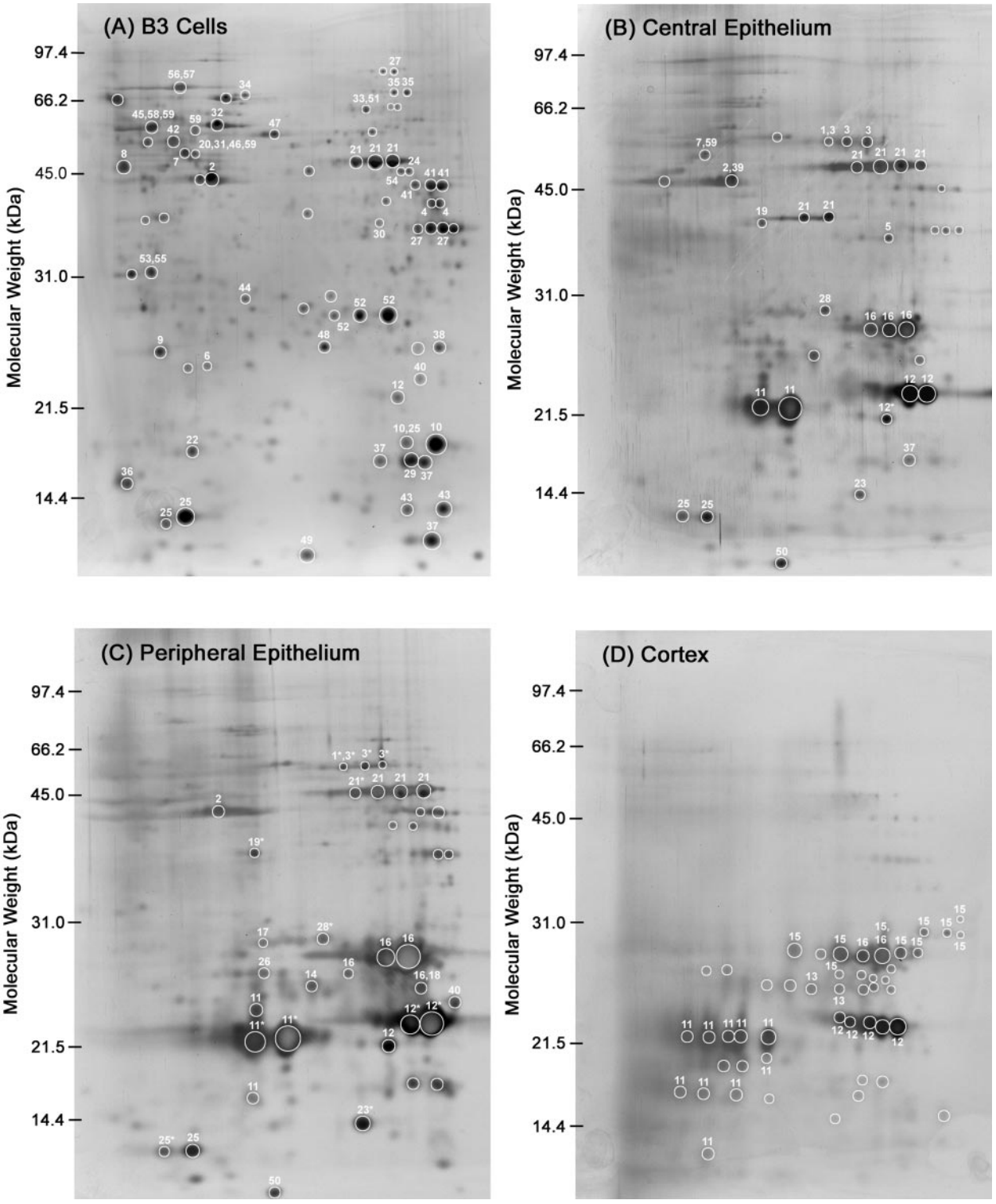

FIGURE 2. Identical with Figure 1, except proteins excised for analysis and identification by MS are *circled*. The names of proteins successfully identified by mass spectrometry are *numbered* and listed in Table 1. Numbers of proteins marked with *asterisks* were identified based on their position and comparison to proteins identified from the corresponding central (**B**) or peripheral (**C**) epithelium gel. (**A**) HLE B-3 cells; (**D**) cortical fiber tissue.

Taxon-specific crystallins in both invertebrates and vertebrates are related to aldehyde dehydrogenase. ω-Crystallin, related to vertebrate ALDH class 1/2, is present in squid, octopus, and scallop.[32–35] η-Crystallin, also related to ALDH class 1/2, is the major crystallin in the elephant shrew lens.[36,37] High levels of ALDH3 have also been found in the cornea.[38,39] ALDH1 reportedly functions to oxidize retinaldehyde to retinoic acid. However, it may also function in human lens as a UV filter due to its binding to NADH.[37]

Lens epithelium also contained an abundance of galectin-1, S100, and fatty acid binding protein 5. Galectin-1 is a member of a large family of β-galactoside binding proteins. Another

Table 1. Identification of Proteins in Cultured HLE B-3 Cells, Donor Lens Central and Peripheral Epithelium, and Cortex

Protein	Gi Number†	B3 Cells	Central	Periphery	Cortex
3-Phosphoglycerate dehydrogenase	1474596		1	1*	
Actin, gamma	71625	2	2	2	
Aldehyde dehydrogenase 1A1	118495		3	3*	
Aldolase A	229674	4			
Annexin I	4502101		5		
ATP synthase, F0 complex, subunit d	5453559	6			
ATP Synthetase beta chain	114549	7	7		
Calumenin	4502551	8			
Chromobox homolog 1	5803076	9			
Cofilin I	5031635	10			
Crystallin, alpha A	4503055		11	11	11
Crystallin, alpha B	4503057	12	12*	12*	12
Crystallin, beta A3	12056461				13
Crystallin, beta A4	4503059			14	
Crystallin, beta B1	4503061				15
Crystallin, beta B2	299263		16	16	16
Crystallin, beta B3	4758074			17	
Crystallin, gamma S	8922120			18	
Dimethylarginine dimethylaminohydrolase	6912328		19	19*	
Dynactin 2	5453629	20			
Enolase, alpha/tau-crystallin	4503571	21	21	21*	
Eukaryotic translation initiation factor 5A	4503545	22			
Fatty acid binding protein 5 (E)	4557581		23	23*	
Fumarate hydratase	19743875	24			
Galectin-1, beta-galactoside binding lectin	4504981	25	25	25*	
Glutathione S-transferase chain A	2914230			26	
Glyceraldehyde-3-phosphate dehydrogenase	7669492	27			
Heat shock 27-kDa protein (HSP27)	4504517		28	28*	
Heat shock 70-kDa protein 5	16507237	29			
Heat shock 70-kDa protein 8 isoform 1	5729877	30			
Heat shock 70-kDa protein binding protein	19923193	31			
Heat shock protein, 60-kDa mitochondrial precursor	129379	32			
Lamin A/C	125962	33			
Mitochondrial stress-70 protein	21264428	34			
Myc far upstream element binding protein	1082624	35			
Myosin light chain alkali, smooth muscle isoform	127148	36			
Peptidylprolyl isomerase A	10863927	37	37		
Peroxiredoxin 1	4505591	38			
Phakinin	4502995		39		
Phosphatidylethanolamine binding protein	4505621	40		40	
Phosphoglycerate kinase I	4505763	41			
Poly(rC) binding protein 1	5453854	42			
Profilin I	1943532	43			
Prohibitin	4505773	44			
Protein disulfide isomerase/prolyl 4-hydroxylase beta	20070125	45			
Protein disulfide isomerase-related protein	5031973	46			
Protein disulfide-isomerase	1085373	47			
RNA binding protein regulatory subunit	6005749	48			
S100 calcium binding protein A11	5032057	49			
S100 calcium binding protein A4	4506765		50	50	
Stress induced phosphoprotein I	5803181	51			
Triosephosphate isomerase I	4507645	52			
Tropomyosin 4	4507651	53			
Tropomyosin beta chain	136090	54			
Tropomyosin, fibroblast	88935	55			
Tubulin beta 2	5174735	56			
Tubulin beta 5	7106439	57			
UV excision repair protein RAD23 homolog B	4506387	58			
Vimentin	4507895	59	59		

* Proteins identified from in-gel digests of spots from 2-DE gels shown in Figures 1 and 2. Each protein was given a single number, corresponding to the labeled spots in Figure 2.

† Gi numbers are unique identifiers for protein sequences cataloged in a database accessed from the National Center for Biotechnology Information.

‡ Due to the similarity of 2-D gels from central and peripheral epithelium, the indicated spots were identified based on their similar position to the identified protein in the corresponding gel (proteins identified by position are denoted by asterisks in Figs. 2B, 2C).

member of this family which has also been described in lens is galactin-3,[40] and the related protein GRIFIN.[41,42] As in other tissues, galactin-1 may function in lens epithelium as a regulator of cell growth, differentiation, and apoptosis, as well as playing a role in cell adhesion.[43] Two distinct forms of S100 calcium-binding proteins were found in cultured lens epithe-

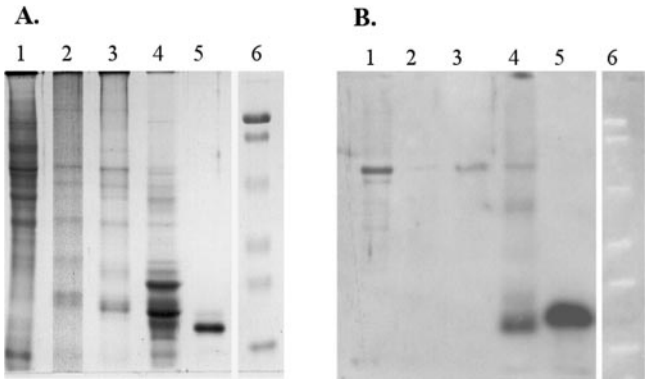

FIGURE 3. γ-Crystallin was probed as a measure of the presence of fiber cells in dissected donor capsule epithelium. (**A**) Coomassie-stained gel. (**B**) Anti-γ-crystallin Western blot. *Lane 1*: HLE B-3 cells (90 μg); *lane 2*: central region of human donor lens epithelial cells (24 μg); *lane 3*: peripheral region of human donor lens epithelial cells (24 μg); *lane 4*: cortical fiber region of human donor lens (24 μg); *lane 5*: purified calf lens γ-crystallin (12 μg); *lane 6*: six molecular weight markers (104, 81, 47.7, 34.6, 28.3, and 19.2 kDa).

lium (S100A11) and freshly dissected epithelium (S100A4). The S100 family proteins are calcium-binding proteins containing EF-hand structures that function to mediate a large number of cellular processes in a manner similar to calmodulin.[44] Whereas the role of S100A11 in lens is unknown, the protein has been widely implicated in metastasis in a wide variety of cancers.[45] Fatty acid binding protein-E (FABP-E) has been previously described in mouse[46] and bovine lenses.[47] Its concentration was reported to increase during differentiation in bovine lens. This finding was supported by the present study, because peripheral epithelium contained more of the protein than central epithelium (Fig. 2B, 2C, spot 23). The higher concentration in differentiating cells may be required for uptake of fatty acids to allow higher rates of membrane synthesis during fiber cell elongation.

The use of cell culture is an essential tool of cellular and molecular biology. Continuous cell culture facilitates studies ranging from manipulation of the genome and the regulation of gene expression to the production of new pharmaceuticals. An added benefit of cell culture is the reduction of animal experimentation, especially in difficult to isolate cells, such as found in lens capsule epithelium. Transformed HLE cells, such as HLE B-3 cells, have and will continue to provide the only viable means to study many aspects of lens biology. Although the majority of the proteins observed in HLE B-3 cells were not observed in noncultured cells, we cannot exclude the possibility that the high abundance of αA-, αB-, and βB2-crystallins in the noncultured cells may have masked the detection of minor components. This is a common problem encountered when analyzing cellular proteomes using 2-DE.[48] However, the absence of the high-abundance proteins αA-, αB-, βB2-crystallin, and aldehyde reductase from HLE B-3 cells suggests that caution should be used when extrapolating findings using these and other transformed HLE cells to study the intact lens.

Extracellular signaling molecules, such as FGF-2, are capable of regulating differentiation and expression of crystallins in cultured lens epithelial cell explants.[49] Additional experiments are needed to explore how supplementation of growth medium with various signaling molecules alters both differentiation and protein composition of lens epithelial cells. Further experiments to identify less abundant proteins in human lens epithelium would also be of great benefit.

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