Molecular Vision 2006; 12:1-14 <http://www.molvis.org/molvis/v12/a1/>
Received 23 May 2005 | Accepted 7 October 2005 | Published 5 January 2006

Gene expression profile of cultured adult compared to immortalized human retinal pigment epithelium

Hui Cai, Lucian V. Del Priore

Department of Ophthalmology, Harkness Eye Institute, Columbia University, New York, NY

Purpose: ARPE19 is a spontaneously immortalized cell line of human retinal pigment epithelium (RPE) that is used widely to draw inferences about the behavior of adult human RPE (ahRPE). We used DNA microarray analysis to compare the gene expression profiles of these two cell types.

Methods: Second-passage cultured ahRPE from four human donors (age range 48-82 years) and ARPE19 cultured to confluence in five dishes were used for this DNA microarray study. Total RNA was isolated and first- and second-strand complimentary DNA was synthesized using standard techniques. Biotin-labeled antisense complimentary RNA was produced by an in vitro transcription reaction. Target hybridization, washing, staining, and scanning probe arrays were done following an Affymetrix GeneChip Expression Analysis Manual. Microarray data were normalized and statistical techniques were used to determine the presence or absence of expression of individual genes within ARPE19 and ahRPE, and their relative expression levels.

Results: Hierarchic clustering analysis demonstrated that the gene expression profile of ahRPE and ARPE19 samples cluster into two distinct groups with no discernable overlap. The expression of 5,634±65 gene probes (out of 12,600 on microarray Human U95Av2 chip) was detected in ARPE19 cells compared to 5,580±84 genes in ahRPE cells from four human donor eyes. Thirty-five genes are expressed exclusively in ahRPE and nine genes exclusively in ARPE19 cells. Fifty additional genes have a threefold increase and 40 genes have a threefold decrease in expression level in ahRPE compared to ARPE19. There was no clear difference in the global expression level of genes known to be related to phagocytosis, angiogenesis, or apoptosis.

Conclusions: There are significant differences in the gene expression profile of ahRPE compared to ARPE19, and with some genes exclusively being expressed in one group and other genes being upregulated or downregulated by threefold. Caution should be exercised when generalizing results obtained from ARPE19 to the behavior of ahRPE.

The retinal pigment epithelium (RPE) forms a hexagonal monolayer of cells between the choriocapillaris and outer retina in the human eye. Proper RPE function is necessary to maintain the integrity of the outer blood-retinal barrier and function of photoreceptors. Several significant ophthalmic disorders have been associated with early RPE dysfunction, including age-related macular degeneration, some forms of retinitis pigmentosa, and other peripheral tapetoretinal degenerations [1,2]. Primary and cultured adult human RPE (ahRPE) have been used to study the physiology of the RPE in health and disease and to determine the cellular and molecular events responsible for RPE dysfunction [3-7]. However there are some limitations in studying RPE harvested from human donors, including donor-to-donor variation [8,9]. ARPE19 is a spontaneous immortalized RPE cell line obtained initially from a single human donor; due to its immortality, this cell line has been studied extensively over the last decade to obtain important insights into RPE cell biology [10-13]. Many of the known functions of ahRPE can be performed by ARPE19, including vitamin A metabolism and phagocytosis of outer segment material [10,14].

Despite these facts, there are clearly some differences between ahRPE and ARPE19 that should be considered when results with the immortalized cell line are extrapolated to the behavior of primary or cultured ahRPE. For example, the promoter of α5 integrin, which is an important molecule for RPE phagocytosis, is regulated differently between these two cell types [15]. Little is known about the overall gene expression profile of these two cell types despite extensive prior study of their properties. In the present study, we use microarray techniques to compare the gene expression profile of ahRPE to that of ARPE19, and demonstrate that there are some significant differences between these two cell types. This study provides an important reference database for subsequent studies in which the results of studies on ARPE19 are extrapolated to RPE.

METHODS

Preparation of adult human retinal pigment epithelium cultures: Human eyes obtained from the National Disease Research Interchange (NDRI, Philadelphia, PA) were processed within 48 h of donor death. The ages and other information regarding donor eyes are shown in Table 1. Four samples from human donor eyes were used; given the expense and avail-

Correspondence to: Lucian V. Del Priore, MD, PhD, Robert L. Burch III Scholar, Department of Ophthalmology, Harkness Eye Institute of Columbia University, 635 West 165th Street, New York, NY; Phone: (212) 305-2923; FAX: (212) 342-1724; email: ldelpriore@yahoo.com

ability of human tissue, small sample sizes have been used in the past to generate data on gene expression within human tissue [16-18]. Primary RPE cell cultures were prepared from the posterior poles of the human cadaver eyes as described previously [19]. Upon receipt in the laboratory, eyes were cleaned of extraocular tissue. The anterior segment structures, vitreous, and retina were removed, leaving an eyecup with RPE on the inner surface. For these studies, 500,000 primary ahRPE cells were collected with trypsin from each pair of globes harvested from four individual human donors (age range 48 to 82 years, Table 1). The cells were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C and maintained in Dulbecco's modified Eagle's medium (DMEM H16; Gibco BRL, Carlsbad, CA) supplemented with 15% fetal bovine serum (FBS; Gibco BRL), 100 IU/ml penicillin G, 100 mg/ml streptomycin, 5 mg/ml gentamicin, 2.5 mg/ml amphotericin B and 1 ng/ml human recombinant basic fibroblast growth factor (bFGF, Gibco BRL) in a 35x10 mm culture dish (Becton Dickinson Labware, Franklin Lakes, NJ) to promote RPE cell growth. The medium was changed every other day. Cells became confluent in about 14 days and confluent cultures were passaged by trypsinization to a 60 mm culture dish (Becton Dickinson Labware). For the microarray analysis about 10^6 RPE cells were harvested from second passage cultures before synchronization by serum depletion and bFGF removal for 24 h and total RNA was isolated for the DNA microarray study.

Cytokeratin labeling: Cells were stained using a pancytokeratin antibody to verify that all cells were of epithelial origin [20,21]. For this purpose, ahRPE cells in a culture dish were rinsed in phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 30 min, and washed again with PBS. The cells were treated for 1 h at room temperature with 3% bovine serum albumin (Sigma Chemical, St. Louis, MO) in PBS to block nonspecific binding sites. The cells were then incubated at 37 °C for 1 h with an fluorescein-isothiocyanate (FITC)-conjugated monoclonal antipan cytokeratin antibody to cytokeratins 5, 6, and 8 (Sigma). The cells were washed thrice with PBS and examined under a fluorescence microscope. An irrelevant isotypic IgG primary antibody (antihuman von Willebrand antibody, Sigma) coupled with an FITC-conjugated secondary antibody was also used and showed no background staining. All of the harvested cells were positive for pancytokeratin indicating the cells were of epithelial origin.

Preparation of immortalized ARPE19: Immortalized human RPE (ARPE19) obtained from ATCC (Manassas, VA) were cultured and propagated in DMEM (Gibco BRL) containing 10% FBS, 100 IU/ml penicillin G, 100 µg/ml streptomycin, 100 ug/ml gentamicin, 2.5 µg/ml amphotericin B (Gibco BRL). The cells were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C, and the culture medium was changed every other day. Cultures were plated in 60 mm tissue culture-treated dishes. Five dishes of 500,000 ARPE19 were cultured for about 10 days to confluence. Cells were synchronized by serum depletion for 24 h, and total RNA was isolated for the DNA microarray study.

Isolation of total RNA from RPE cells: RPE (either ARPE19 or ahRPE) were harvested by trypsinization, and RNA was isolated and purified using a Qiagen RNeasy Mini Kit (Qiagen Co., Valencia, CA) according to manufacturer's instructions. RPE cells (about 10^6 cells) were disrupted, and total RNA was isolated using a QIA shredder and RNeasy Mini Kit. Briefly, 600 µl of lysing buffer (RLT) was added to cells in a 1.5-ml microfuge tube and cell lysate was loaded onto a QIA shredder spin column and spun for 2 min at 13,000 rpm. The homogenized lysate was then mixed with 600 µl of 70% ethanol and applied to an RNeasy mini spin column and centrifuged for 15 s at 13,000 rpm. Next 700 µl of buffer RW1 and buffer RPE were added and spun sequentially for washing twice. Then 60 µl RNase-free water was used to elute total RNA from RNeasy column. All total RNA used in the experiments were relatively pure (A260/A280>1.9). Total RNA was stored at -80 °C for later use.

DNA microarray experiments: Affymetrix U95Av2 chips (Affymetrix Inc., Santa Clara, CA) were used in the experiments. Each chip contained 12,600 human gene probes and has 1,090 RPE-related gene probes, and gene probes for many

Table 1. Adult human retinal pigment epithelium cells donor information

Tissues	Death to enucleation time (h)	Enucleation to ahRPE extraction time (h)	Age (years)	Gender	Cause of death
Sample 1	7	23	48	F	cardiac arrest
Sample 2	3.5	18	68	M	cardiomyopathy
Sample 3	7	19	75	M	interstitial cystitis hemorrhage
Sample 4	7	17	82	M	myocardial

Human eyes (age range 48 to 82 years) obtained from the National Disease Research Interchange were enucleated within 7 h and ahRPE were processed within 30 h of donor death.

other genes related to basic cell functions such as cell proliferation and differentiation. The advantages of using such an array include the large number of genes that are measured, and that no selection bias arises from the use of pathway-specific arrays that are designed to look at proliferation genes, apoptosis genes, etc.

A T7-$(dT)_{24}$ oligomer, superscript reverse transcriptase II and DNA Polymerase I (Gibco BRL) were used for first-strand and second-strand cDNA synthesis using total RNA as templates. Double-stranded cDNA was cleaned with Phase Lock Gels-Phenol/Chloroform extraction and ethanol precipitation. Biotin-labeled antisense cRNA was produced by an in vitro transcription reaction (ENZO BioArray High Yield RNA Transcript Labeling Kit; Affymetrix Inc.) and incubated with fragmentation buffer (Tris-acetate, KOAc and MgOAcat; 94 °C for 35 min). Target hybridization, washing, staining, and scanning probe arrays were done following an Affymetrix GeneChip Expression Analysis Manual.

Quality controls, definitions of gene presence or absence, and statistical analysis: For quality control, the U95Av2 DNA microarray chips used includes 20 housekeeping gene probes to measure the consistency of the hybridization signals from their 3', middle, and 5' fragment of these mRNA coding regions [22]. The definitions of presence and absence of gene expression are defined with Affymetrix GCOS 1.2 statistical algorithm [23] and further filtered, with “presence” defined as a scan densitometry reading of >50 and “absence” defined as a scan densitometry reading of <50. For comparison of the different levels of gene expression between ahRPE and ARPE19, the changes were considered to be significant if there was a greater than threefold increases or decreases in the expression level of these two cell types and if the changes were statistically different ($p<0.01$, student’s t-test). Gene expression analyses, including global normalization and scaling were performed using the Affymetrix GCOS 1.2, Array Assist 3.01 (Stratagene, La Jolla, CA) software.

RESULTS

Quality control assessment: After 10 days in culture, ahRPE cells appeared healthy and confluent (data not shown). All nine DNA chips passed quality control using the hybridization signals from 3', middle, and 5' fragment of mRNA of 20 housekeeping genes coded in Affymetrix DNA chips.

Hierarchic clustering analysis: Clustering analysis is a statistical technique that is used to sort heterogeneous samples into several distinct clusters; samples within the cluster have more of a relationship to one another than samples from dif-

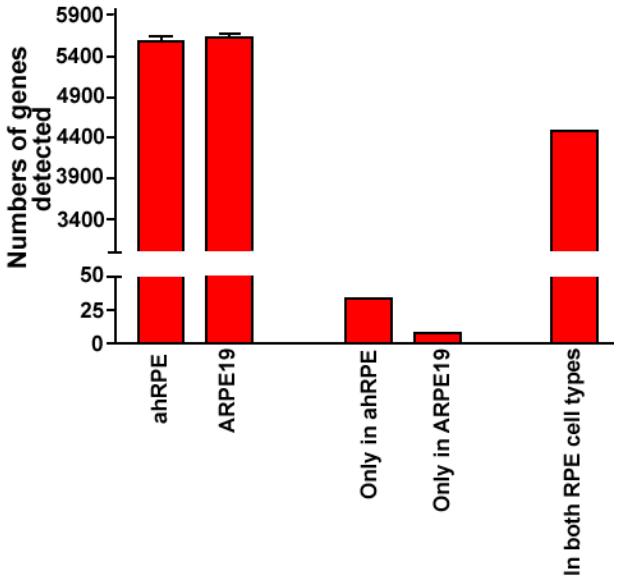

Figure 2. Number of genes detected in ahRPE and/or ARPE19 cells. Using Affymetrix U95Av2 chips, which contains 12,600 genes, there are 5,580 genes expressed in ahRPE cells and 5,634 genes expressed in ARPE19 cells (t-test, p=0.312). Out of these 4,479 genes are expressed in both cell types, while 35 and 9 genes are exclusively expressed in ahRPE or ARPE19 cells, respectively.

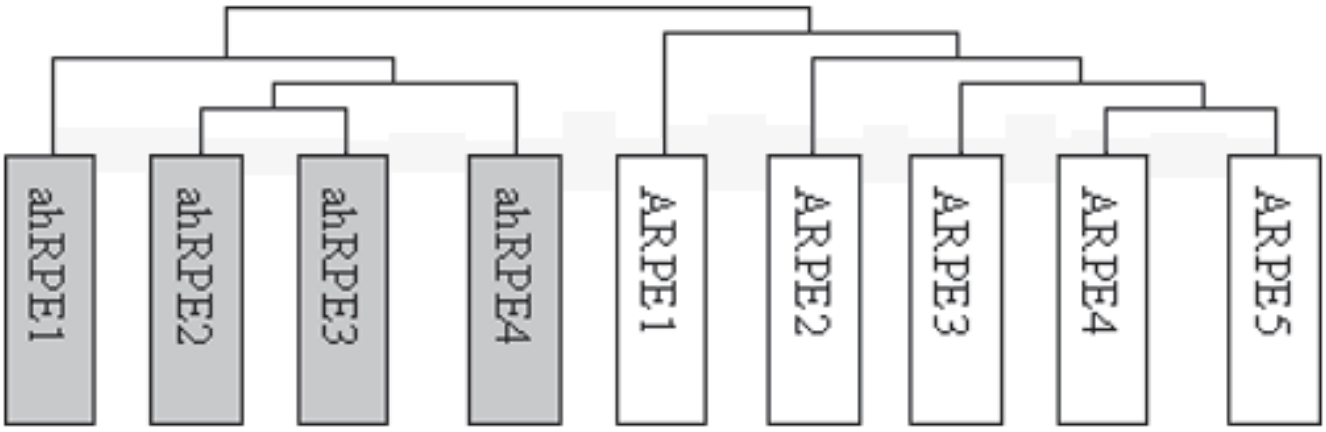

Figure 1. Hierarchic clustering analysis of human adult RPE and ARPE19 samples. As demonstrated, the gene expression profile of four samples from the adult human RPE (shown in shaded boxes) and five samples from ARPE19 cluster into two distinct groups with no discernible overlap.

Table 2. Genes expressed in adult human RPE (ahRPE) but not in ARPE19 cells

Probe ID	Gene title	Symbol	ahRPE	ARPE19	Gene functions
1120_at	glutathione S-transferase M3 (brain)	GSTM3	112.8	31.2	metabolism
1139_at	guanine nucleotide binding protein (G protein), α 13	GNA13	50.4	9.8	cell motility; signal transduction
1712_s_at	mitogen-activated protein kinase kinase 4	MAP2K4	53.5	17.0	proteinphosphorylation; signal transduction
2041_i_at	v-abl Abelson murine leukemia viral oncogene homolog 1	ABL1	123.9	35.8	regulation of cell cycle; induction of apoptosis
32076_at	Down syndrome critical region gene 1-like 1	DSCR1L1	87.0	22.1	calcium-mediated signaling
32349_at	annexin A10	ANXA10	134.5	18.5	negative regulation of coagulation
32818_at	tenascin C (hexabrachion)	TNC	90.8	9.8	cell adhesion
33267_at	ATPase, aminophospholipid transporter (APLT)	ATP8A1	115.9	32.6	cation transport; metabolism
33505_at	retinoic acid receptor responder (tazarotene induced) 1	RARRES1	134.1	23.6	negative regulation of cell proliferation
33803_at	thrombomodulin	THBD	92.4	23.7	blood coagulation
34363_at	selenoprotein P, plasma, 1	SEPP1	68.0	5.7	response to oxidative stress
34591_at*	deiodinase, iodothyronine, type III	DIO3	512.1	13.1	thyroxine 5'-deiodinase activity
34666_at	superoxide dismutase 2, mitochondrial	SOD2	324.7	33.2	superoxide metabolism
34823_at	dipeptidylpeptidase 4 (CD26)	DPP4	90.6	10.3	proteolysis; immune response
35663_at	neuronal pentraxin II	NPTX2	93.2	3.7	synaptic transmission
35885_at	ubiquitin specific protease 9	USP9Y	65.6	5.0	ubiquitin-dependent protein catabolism
35980_at	phospholipase C, β 1 (phosphoinositide-specific)	PLCB1	67.7	9.2	regulation of cell cycle; lipid catabolism
36312_at	serine (or cysteine) proteinase inhibitor	SERPINB8	120.6	28.8	serine-type endopeptidase inhibitor activity
36650_at	cyclin D2	CCND2	113.9	5.5	regulation of cell cycle; cytokinesis
37363_at	metastasis suppressor 1	MTSS1	68.8	8.3	cell motility; cell adhesion; neurogenesis
37402_at*	ribonuclease, RNase A family, 1 (pancreatic)	RNASE1	349.7	11.8	endonuclease activity
38323_at	carboxypeptidase, vitellogenic-like	CPVL	111.3	4.4	proteolysis and peptidolysis

TABLE 2, CONTINUED.

Probe ID	Gene title	Symbol	ahRPE	ARPE19	Gene functions
38327_at	silver homolog (mouse)	SILV	181.6	39.2	melanin biosynthesis from tyrosine
38355_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	DDX3Y	218.7	6.9	nucleic acid binding hydrolase activity
38407_r_at*	prostaglandin D2 synthase 21 kDa (brain)	PTGDS	1056.6	10.3	prostaglandin biosynthesis; transport
38469_at	transmembrane 4 superfamily member 3	TM4SF3	247.5	11.3	protein amino acid glycosylation
38528_at	acetyl-Coenzyme A carboxylase α	ACACA	118.3	44.9	fatty acid biosynthesis; metabolism
40097_at	eukaryotic translation initiation factor 1A, Y-linked	EIF1AY	53.0	4.2	protein biosynthesis; translational initiation
40583_at	protein tyrosine phosphatase, receptor type, J	PTPRJ	55.7	10.5	protein dephosphorylation; cell-cell signaling
40899_at*	keratin 19	KRT19	632.1	6.1	structural molecule; intermediate filament
41089_at	ATPase, Ca++ transporting, plasma membrane 4	ATP2B4	87.0	20.4	calcium ion transport; metabolism
41373_s_at	microtubule-associated protein 1B	MAP1B	69.2	7.0	muscle development
434_at	H1 histone family, member 0	H1F0	73.3	6.0	nucleosome assembly
759_at	prostaglandin I2 (prostacyclin) synthase	PTGIS	134.3	19.4	electron transport; lipid metabolism
33339_g_at	signal transducer and activator of transcription 1, 91 kDa	STAT1	187.9	19.1	regulation of cell cycle; caspase activation

Data were generated from Affymetrix U95Av2 DNA microarray analysis of four ahRPE and five ARPE19 samples. Numbers shown in ahRPE and ARPE19 columns are the mean values of DNA microarray chip densitometry scan signals. Asterisk (*) indicates genes within 2,500 most abundant gene list (data not shown).

ferent clusters [24,25]. Hierarchical clustering yields a branching or tree diagram with the branches indicating the relationship of samples within the cluster to other samples both within and outside the clusters [26]. Hierarchical cluster analysis of the gene expression profile of the cells demonstrates that the gene expression profile of ahRPE and ARPE19 cluster into two distinct groups with no discernable overlap (Figure 1).

Expression profiles of adult RPE and ARPE19 cells: The expression of 5,634±65 gene probes (range: 5,554 to 5,726) out of 12,600 gene probes on microarray Human U95Av2 chip is detected in five ARPE19 cell samples, in comparison to detection of only 5,580±84 gene probes (range: 5,508 to 5,695) in ahRPE cells from four human donor eyes (Figure 2). More genes were detected among ARPE19 cells but this difference was not statistically significant (student t-test, p=0.312). To minimize the ambiguity for the data analyzed, the study does not include any genes whose expression levels are defined as marginal presence.

Genes detected exclusively in either cell type: We then determined the list of genes expressed in only one cell type, using stringent criteria in which genes of interest were detected as present in all samples from one cell type and not present in any of the samples from the other cell type, and in which the expression level was consistently greater than 50 in densitometry readings. Thirty-five genes were expressed only in ahRPE and nine genes were expressed only in ARPE19 cells (Figure 2). The list of these genes and their reported functions are in Table 2 and Table 3. Of the 2,500 most abundantly expressed genes on Affymetrix U95A chip in adult RPE cells, four genes were not expressed in ARPE19 (Table 2). As for

the nine uniquely expressed ARPE19 genes, none were contained within the 2,500 most abundant RPE gene list. There were 2,437 (97.5%) genes present within the list of the 2,500 most abundant genes in each group.

Different expression levels: In addition to analysis of present or absent genes in ahRPE or ARPE19, the relative expression levels of the genes present in all nine ahRPE and ARPE19 samples (4,479 genes) are also compared. After normalization of all nine samples there are 50 genes in ahRPE samples whose expression levels are increased >3 fold compared to ARPE19 (Table 4). There are 40 genes whose expression levels in ahRPE are decreased >3 fold compared to ARPE19 (Table 5). When we applied the same criteria to the list of the 2,500 most abundant genes, there are 35 genes expressed at higher levels in adult RPE and seven genes expressed higher levels in ARPE19 cells (Table 4, Table 5). We also compared the expression levels of genes related to known RPE functions [27]. We were able to identify a total of 23 genes involved in phagocytosis, 142 for neurogenesis, and 40 for angiogenesis on Affymetrix U95Av2 DNA microarray chip [27]. Interestingly, none of these genes showed a differential expression between these two cell types, and thus did not show up in our differentially expressed gene list, suggesting that expression of genes responsible for major RPE functions is similar between ahRPE and ARPE19.

DISCUSSION

ARPE19 is a spontaneously immortalized cell line isolated in the early 1990s that has been used extensively to study RPE cell biology [10,12,15,28]. Our recent literature search on public domain database (PubMED, updated on April 23, 2005) found more than 300 published papers regarding RPE cell biology studies in 2004 alone, and approximately 1/8 of these employed ARPE19. In many ways ARPE19 exhibit behavior that is similar to the behavior of adult human RPE. For example, ARPE19 form polarized epithelial monolayers with tight junctions and show barrier properties. Both types of RPE cells express RPE-specific markers CRALBP and RPE65, and both require the integrin receptor α(v)β5 for the binding and internalization of rod outer segments for phagocytosis [10,14]. Despite the extensive use of ARPE19 to understand RPE cell biology, to our knowledge the similarities and differences between the gene expression profiles of these cell types has not been characterized comprehensively elsewhere. Our study demonstrates that the global gene expression profiles of ARPE19 and ahRPE clusters into two distinct groups with no

Table 3. Genes expressed in ARPE19 but not in adult human RPE (ahRPE)

Probe ID	Gene title	Symbol	ahRPE	ARPE19	Gene functions
1562_g_at	dual specificity phosphatase 8	DUSP8	27.0	154.9	inactivation of MAPK; protein dephosphorylation
32090_at	nicotinamide nucleotide adenylyltransferase 2	NMNAT2	20.4	65.9	phospholipid biosynthesis; NAD biosynthesis
32753_at	splicing factor 3b, subunit 3, 130 kDa	SF3B3	39.6	73.4	mRNA splicing, protein complex assembly
34801_at	ubiquitin specific protease 52	USP52	49.2	85.4	proteolysis
36458_at	KIAA1018 protein	KIAA1018	45.2	120.0	DNA repair
37580_at	SH3-domain GRB2-like 3	SH3GL3	25.7	50.3	signal transduction
38312_at	olfactomedin-like 2A	OLFML2A	21.4	96.9	latrotoxin receptor activity
39218_at	zinc finger protein 23 (KOX 16)	ZNF23	31.2	68.3	regulation of transcription, DNA-dependent
39590_at	amyloid β (A4) precursor protein-binding	APBA2	39.8	164.0	neurogenesis; protein transport

Data were generated from Affymetrix U95Av2 DNA microarray analysis of four ahRPE and five ARPE19 samples. Numbers shown in ahRPE and ARPE19 columns are the mean values of DNA microarray chip densitometry scan signals. None of these nine genes are within the 2,500 most abundant gene list (data not shown).

Table 4. Genes within adult human RPE (ahRPE) upregulated genes >three fold compared to ARPE19

Probe ID	Gene title	Symbol	ahRPE	ARPE19	ARPE19/ ahRPE	p value	Gene functions
36533_at*	prostaglandin I2 (prostacyclin) synthase	PTGIS	1254.8	71.2	17.6	0.000023	electron transport; lipid metabolism
41294_at*	keratin 7	KRT7	4157.1	239.2	17.4	0.000250	cytoskeleton organization and biogenesis
34265_at*	secretory granule, neuroendocrine protein 1 (7B2 protein)	SGNE1	305.6	45.3	6.7	0.000000	protein folding; intracellular protein transport
32434_at	myristoylated alanine-rich protein kinase C substrate	MARCKS	328.3	50.8	6.5	0.000000	cell motility
38957_at	doublecortin and CaM kinase-like 1	DCAMKL1	260.0	43.4	6.0	0.001277	protein phosphorylation; endosome transport
41505_r_at	v-maf musculoaponeurotic fibrosarcoma oncogene homolog	MAF	133.3	23.6	5.7	0.001446	regulation of transcription
39927_at	Rho GTPase activating protein 5	ARHGAP5	120.4	21.4	5.6	0.000301	cell adhesion
40367_at	bone morphogenetic protein 2	BMP2	245.6	49.9	4.9	0.000051	skeletal development; cell-cell signaling
40455_at*	KIAA0830 protein	KIAA0830	609.6	125.1	4.9	0.000021	nucleic acid binding; endonuclease activity
865_at*	ribosomal protein S6 kinase, 90 kDa, polypeptide 3	RPS6KA3	936.4	194.0	4.8	0.000269	skeletal development; protein phosphorylation
40035_at*	kallikrein 11	KLK11	1023.1	213.3	4.8	0.000000	proteolysis and peptidolysis
40493_at*	CD44 antigen	CD44	2812.0	604.9	4.6	0.000027	cell adhesion; cell-matrix adhesion
33103_s_at	adducin 3 (γ)	ADD3	115.8	25.1	4.6	0.000006	calmodulin binding
38634_at*	retinol binding protein 1, cellular	RBP1	1152.3	250.5	4.6	0.000012	vitamin A metabolism; transport
33236_at*	retinoic acid receptor responder (tazarotene induced) 3	RARRES3	406.3	90.8	4.5	0.000080	growth regulatory protein; tumor suppressor
31720_s_at*	fibronectin 1	FN1	1496.7	341.1	4.4	0.000050	cell adhesion; cell migration
33849_at	pre-B-cell colony enhancing factor 1	PBEF1	150.5	34.6	4.4	0.003907	signal transduction; cell proliferation regulation

TABLE 4, CONTINUED.

Probe ID	Gene title	Symbol	ahRPE	ARPE19	ARPE19/ ahRPE	p value	Gene functions
38138_at*	S100 calcium binding protein A11 (calgizzarin)	S100A11	3690.0	848.9	4.3	0.000001	negative regulation of cell proliferation
34642_at*	tyrosine 3-monooxygenase activation protein	YWHAZ	2301.6	544.5	4.2	0.000003	protein domain specific binding
266_s_at	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	CD24	129.5	32.0	4.0	0.005621	immune response
40227_at*	discoidin, CUB and LCCL domain containing 2	DCBLD2	1007.5	260.5	3.9	0.000037	cell adhesion; wound healing
1456_s_at	interferon, γ-inducible protein 16	IFI16	118.2	31.1	3.8	0.000007	immune response; cell proliferation
32533_s_at*	vesicle-associated membrane protein 5 (myobrevin)	VAMP5	577.1	155.0	3.7	0.000002	myogenesis; vesicle-mediated transport
40500_at	NDRG family member 4	NDRG4	290.6	78.8	3.7	0.000398	response to stress; cell differentiation
1991_s_at*	mitogen-activated protein kinase-activated protein kinase 3	MAPKAPK3	1195.9	329.0	3.6	0.000009	protein phosphorylation; response to stress
35726_at*	S100 calcium binding protein A2	S100A2	1584.2	437.1	3.6	0.000071	calcium ion binding
36262_at*	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	GNS	1089.7	303.0	3.6	0.000969	glycosaminoglycan catabolism; metabolism
33863_at*	hypoxia upregulated 1	HYOU1	4796.3	1336.2	3.6	0.000001	protein folding; response to stress
672_at*	serine (or cysteine) proteinase inhibitor	SERPINE1	2268.9	676.6	3.4	0.000211	blood coagulation
35769_at*	G protein-coupled receptor 56	GPR56	1484.9	445.4	3.3	0.000580	neuropeptide signaling pathway; metabolism
37628_at*	monoamine oxidase B	MAOB	289.5	87.3	3.3	0.000175	electron transport
37319_at*	insulin-like growth factor binding protein 3	IGFBP3	1438.0	437.2	3.3	0.008582	cell growth and apoptosis regulation
34886_at*	radixin	RDX	402.1	123.6	3.3	0.000024	cytoskeletal anchoring
38500_at*	CGI-109 protein	CGI-109	611.8	190.3	3.2	0.000012	intracellular protein transport
33339_g_at	signal transducer and activator of transcription 1, 91 kDa	STAT1	111.9	35.0	3.2	0.000024	regulation of cell cycle; caspase activation

TABLE 4, CONTINUED.

Probe ID	Gene title	Symbol	ahRPE	ARPE19	ARPE19/ ahRPE	p value	Gene functions
39373_at*	fatty acid desaturase 1	FADS1	478.7	150.3	3.2	0.001098	fatty acid biosynthesis; cell-cell signaling
37391_at*	cathepsin L	CTSL	3573.0	1126.1	3.2	0.000572	proteolysis and peptidolysis
1034_at*	tissue inhibitor of metalloproteinase 3	TIMP3	1293.7	412.0	3.1	0.000456	visual perception; induction of apoptosis
37405_at*	selenium binding protein 1	SELENBP1	995.0	317.0	3.1	0.000836	selenium binding
160020_at*	matrix metalloproteinase 14 (membrane-inserted)	MMP14	2332.0	748.7	3.1	0.000168	proteolysis and peptidolysis
35992_at*	musculin (activated B-cell factor-1)	MSC	576.5	186.0	3.1	0.000157	regulation of transcription
31783_at*	renin binding protein	RENBP	321.7	103.8	3.1	0.000111	mannose metabolism
40848_g_at	flavoprotein oxidoreductase MICAL2	MICAL2	263.6	85.4	3.1	0.000186	electron transport; metabolism
33989_f_at*	testis enhanced gene transcript (BAX inhibitor 1)	TEGT	3358.8	1092.8	3.1	0.000171	negative regulation of apoptosis
40913_at*	ATPase, Ca++ transporting, plasma membrane 4	ATP2B4	734.8	239.6	3.1	0.000002	calcium ion transport; metabolism
38837_at	hypothetical protein DJ971N18.2	DJ971N18.2	174.9	57.2	3.1	0.000004	electron transport
33372_at*	RAB31, member RAS oncogene family	RAB31	830.0	271.3	3.1	0.000467	small GTPase mediated signal transduction
2092_s_at	secreted phosphoprotein 1	SPP1	93.7	30.7	3.1	0.000096	anti-apoptosis; cell-matrix adhesion
41739_s_at	caldesmon 1	CALD1	224.9	73.8	3.0	0.000160	muscle contraction; muscle development
40433_at*	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	GNS	575.3	191.0	3.0	0.000111	glycosaminoglycan catabolism; metabolism

Data were generated from Affymetrix U95Av2 DNA microarray analysis of four ahRPE and five ARPE19 samples. Numbers shown in ahRPE and ARPE19 columns are the mean values of DNA microarray chip densitometry scan signals. Numbers shown in ahRPE/ARPE19 column are fold-changes comparing ahRPE gene expression level with ARPE19. Numbers in p value column are from student t-test. Asterisk indicates genes within 2,500 most abundant gene list (data not shown).

Table 5. Genes within ARPE19 upregulated genes >three fold compared to adult human RPE (ahRPE)

Gene title	Symbol	ARPE19	ahRPE	ARPE19/ ahRPE	p value	Gene functions
N-myristoyltransferase 2	NMT2	594.9	106.6	5.6	0.000001	N-terminal protein myristoylation; protein-lipoylation
ectonucleotide pyrophosphatase/phosphodiesterase 2	ENPP2	1604.2	319.5	5.0	0.000004	phosphate metabolism; cell motility
interleukin 18 (interferon-γ-inducing factor)	IL18	710.7	157.9	4.5	0.000572	angiogenesis; immune response; apoptosis; cell adhesion
V-erb-a erythroblastic leukemia viral oncogene homolog 4	ERBB4	249.5	57.6	4.3	0.000001	protein phosphorylation; cell proliferation
IGF-II mRNA-binding protein 3	IMP-3	440.5	108.1	4.1	0.000273	RNA processing; protein biosynthesis; morphogenesis
glypican 3	GPC3	695.2	173.8	4.0	0.001307	morphogenesis
ferredoxin reductase	FDXR	1152.5	289.2	4.0	0.000003	electron transport; steroid biosynthesis
v-jun sarcoma virus 17 oncogene homolog (avian)	JUN	345.9	88.3	3.9	0.001689	regulation of transcription, DNA-dependent
bromodomain adjacent to zinc finger domain, 2A	BAZ2A	538.9	142.3	3.8	0.001987	chromatin remodeling; regulation of transcription
kinesin family member 5C	KIF5C	407.4	108.0	3.8	0.000937	microtubule-based movement
regulator of G-protein signalling 20	RGS20	468.5	126.9	3.7	0.000192	signal transduction
damage-specific DNA binding protein 2, 48 kDa	DDB2	796.8	217.7	3.7	0.000000	nucleotide-excision repair
protein kinase C, β 1	PRKCB1	514.4	141.0	3.6	0.001343	protein phosphorylation; intracellular signaling cascade
phosphoribosyl pyrophosphate synthetase-associated protein 1	PRPSAP1	824.8	230.3	3.6	0.000000	nucleoside metabolism; nucleotide biosynthesis
protein kinase C, α	PRKCA	430.2	122.2	3.5	0.000008	protein phosphorylation; induction of apoptosis
chromosome 6 open reading frame 111	C6orf111	94.8	27.0	3.5	0.000054	unknown
cystathionine-β	CBS	222.7	64.3	3.5	0.000249	amino acid biosynthesis; metabolism

TABLE 5, CONTINUED.

Gene title	Symbol	ARPE19	ahRPE	ARPE19/ ahRPE	p value	Gene functions
discs, large homolog 1 (Drosophila)	DLG1	113.4	32.8	3.5	0.004625	guanylate kinase activity; cytoskeletal protein binding
heterogeneous nuclear ribonucleoprotein A3	HNRPA3	194.8	56.4	3.5	0.010911	RNA binding; ribonucleoprotein complex
cyclin H	CCNH	1135.8	333.4	3.4	0.000227	regulation protein kinase activity; cell cycle
HERV-H LTR-associating 1	HHLA1	1139.2	335.0	3.4	0.000619	human endogenous retrovirus
actin, α 2, smooth muscle, aorta	ACTA2	1572.9	466.2	3.4	0.000000	muscle development
growth arrest and DNA-damage-inducible, α	GADD45A	725.9	215.7	3.4	0.000204	regulation of protein kinase activity; apoptosis
KIAA0179	KIAA0179	519.2	155.7	3.3	0.000021	rRNA processing; superoxide metabolism
Purkinje cell protein 4	PCP4	1231.5	372.1	3.3	0.003288	central nervous system development
cytochrome b-245, α polypeptide	CYBA	2015.6	612.7	3.3	0.000000	electron transport; superoxide metabolism
DNA segment on chromosome X and Y	DXYS155E	480.4	146.6	3.3	0.001093	regulation of transcription, signal transduction
angiomotin like 2	AMOTL2	830.0	255.2	3.3	0.000036	regulation of visceral endoderm movement
heterogeneous nuclear ribonucleoprotein A1	HNRPA1	1233.9	381.2	3.2	0.000020	mRNA processing; transport
reelin	RELN	353.7	109.4	3.2	0.000597	cell adhesion; development
cordon-bleu homolog (mouse)	COBL	214.8	66.5	3.2	0.000007	unknown
transforming growth factor, β receptor III	TGFBR3	281.3	87.5	3.2	0.001633	signal transduction; signaling pathway
ataxin 2-like	ATXN2L	920.9	288.4	3.2	0.000103	unknown
Full length insert cDNA YH77E09	---	263.8	84.0	3.1	0.000034	unknown
actin binding LIM protein 1	ABLIM1	537.3	173.3	3.1	0.000096	cytoskeleton organization and biogenesis
aminopeptidase puromycin sensitive	NPEPPS	996.0	323.1	3.1	0.000000	proteolysis and peptidolysis
golgin-67	GOLGIN-67	343.8	112.1	3.1	0.000006	Golgi apparatus

TABLE 5, CONTINUED.

Gene title	Symbol	ARPE19	ahRPE	ARPE19/ ahRPE	p value	Gene functions
amyloid beta precursor protein binding protein 2	APPBP2	252.7	82.8	3.1	0.000021	intracellular protein transport
serine/threonine kinase 6	STK6	252.1	82.8	3.0	0.000732	protein phosphorylation; cell cycle; mitosis

Data were generated from Affymetrix U95Av2 DNA microarray analysis of four ahRPE and five ARPE19 samples. Numbers shown in ahRPE and ARPE19 columns are the mean values of DNA microarray chip densitometry scan signals. Numbers shown in ahRPE/ARPE19 column are fold-changes comparing ahRPE gene expression level with ARPE19. Numbers in p value column are from student t-test. Asterisk indicates genes within 2,500 most abundant gene list.

discernible overlap on the hierarchical clustering analysis. Simultaneously, there is remarkable consistency in the gene expression profile within the ARPE19 subgroup and ahRPE subgroup, respectively, in the distribution and number of genes expressed. A larger number of genes appear expressed in ARPE19 than in ahRPE even though this difference was not statistically significant. There are 35 genes detectable in ahRPE but are missing in ARPE19. A careful consideration of these genes suggests that many of these genes play an important role in the structure and function of the RPE (Table 2). For instances, tenascin, which is expressed in ahRPE but not in ARPE19, has shown to be involved in RPE cell migration [29,30].

Selenoprotein P, an antioxidant protein that protects eyes of experimental animals from oxidative damage, may be important to the development and pathogenesis of age-related macular degeneration [31]. Mitogen-activated protein kinase 4 is involved in the stress-activated kinase signaling pathway that leads to RPE cell death [32]. Guanine nucleotide binding protein (G-protein), retinoic acid receptor responder, and dipeptidylpeptidase 4 are also expressed solely in ahRPE, and the biological effect of the absence of these major regulators of cell proliferation and differentiation in ARPE19 is not known [33-35].

Additional differences in gene expression between these two cell types arise when we compare the absolute expression levels within ARPE19 and ahRPE. Several cell differentiation-related genes [36-38], including NDRG family member 4, interferon γ-inducible protein 16, and CD24 antigens, are upregulated in ahRPE cells. The role of these genes in the proper function of the retina and RPE are not known. It is possible that the low expression levels of the differentiation related genes in ARPE19 compared with ahRPE cells may hamper the ability of ARPE19 to maintain a state of differentiation, which is consistent with the observation that proliferation-related genes are expressed at a higher level in an immortalized cell line [39,40]. Simultaneously, proliferation-related genes, such as serine/threonine kinase 6 and V-erb-a erythroblastic leukemia viral oncogene homolog 4, are upregulated in ARPE19 [41,42]. We noted no differences in the expression levels of known genes involved in phagocytosis, neurogenesis, and angiogenesis on the Affymetrix U95A DNA chip, thus suggesting that genes responsible for these RPE functions are preserved in ARPE19 cell line.

It is known that the gene expression profile of cells can be altered by changes in the surrounding environment, including changes in the culture medium [15], passage number [43], and contact with retinal outer segment [44]. Previous workers have demonstrated that results obtained can also depend upon the platform used to study the gene expression profile [45]. In the current study, we use culture conditions that are similar to those commonly employed to culture RPE, although some caution should be exercised in generalizing these results to other culture conditions or to the expression profile of RPE in vivo.

Basic fibroblast growth factor (bFGF) was removed from the medium by washing 24 h before collecting cultured adult RPE in the experiment. We cannot exclude the possibility that bFGF may have residual effects on the primary RPE culture, but most published data studying primary RPE use bFGF as a supplement.

There are remarkable similarities but significant differences in the gene expression profile of cultured adult and immortalized ARPE cells, and it is important to note that some specific genes are only expressed in one of these two groups. These studies suggest caution should be exercised when generalizing results obtained from ARPE19 to results that would be obtained with adult RPE.

ACKNOWLEDGEMENTS

Supported by the Robert L. Burch III Fund, the Macula Society, the Macula Foundation, the Foundation Fighting Blindness, and unrestricted funds from Research to Prevent Blindness.

REFERENCES

1. Eagle RC Jr. Mechanisms of maculopathy. Ophthalmology 1984; 91:613-25.
2. Jackson GR, Owsley C, Curcio CA. Photoreceptor degeneration and dysfunction in aging and age-related maculopathy. Ageing Res Rev 2002; 1:381-96.
3. Ishida BY, Bailey KR, Duncan KG, Chalkley RJ, Burlingame AL, Kane JP, Schwartz DM. Regulated expression of apolipoprotein E by human retinal pigment epithelial cells. J Lipid Res 2004; 45:263-71.
4. Edwards RB, Brandt JT, Hardenbergh GS. A 31,000-dalton protein released by cultured human retinal pigment epithelium. In-

vest Ophthalmol Vis Sci 1987; 28:1213-8.
5. Tezel TH, Del Priore LV. Serum-free media for culturing and serial-passaging of adult human retinal pigment epithelium. Exp Eye Res 1998; 66:807-15.
6. Del Priore LV, Geng L, Tezel TH, Kaplan HJ. Extracellular matrix ligands promote RPE attachment to inner Bruch's membrane. Curr Eye Res 2002; 25:79-89.
7. Tezel TH, Del Priore LV. Reattachment to a substrate prevents apoptosis of human retinal pigment epithelium. Graefes Arch Clin Exp Ophthalmol 1997; 235:41-7.
8. Garg TK, Chang JY. Oxidative stress causes ERK phosphorylation and cell death in cultured retinal pigment epithelium: prevention of cell death by AG126 and 15-deoxy-delta 12, 14-PGJ2. BMC Ophthalmol 2003; 3:5.
9. Zarbin MA. Analysis of retinal pigment epithelium integrin expression and adhesion to aged submacular human Bruch's membrane. Trans Am Ophthalmol Soc 2003; 101:499-520.
10. Dunn KC, Aotaki-Keen AE, Putkey FR, Hjelmeland LM. ARPE-19, a human retinal pigment epithelial cell line with differentiated properties. Exp Eye Res 1996; 62:155-69.
11. Alizadeh M, Wada M, Gelfman CM, Handa JT, Hjelmeland LM. Downregulation of differentiation specific gene expression by oxidative stress in ARPE-19 cells. Invest Ophthalmol Vis Sci 2001; 42:2706-13.
12. Bejjani RA, BenEzra D, Cohen H, Rieger J, Andrieu C, Jeanny JC, Gollomb G, Behar-Cohen FF. Nanoparticles for gene delivery to retinal pigment epithelial cells. Mol Vis 2005; 11:124-32.
13. King RE, Kent KD, Bomser JA. Resveratrol reduces oxidation and proliferation of human retinal pigment epithelial cells via extracellular signal-regulated kinase inhibition. Chem Biol Interact 2005; 151:143-9.
14. Finnemann SC, Bonilha VL, Marmorstein AD, Rodriguez-Boulan E. Phagocytosis of rod outer segments by retinal pigment epithelial cells requires alpha(v)beta5 integrin for binding but not for internalization. Proc Natl Acad Sci U S A 1997; 94:12932-7.
15. Proulx S, Landreville S, Guerin SL, Salesse C. Integrin alpha5 expression by the ARPE-19 cell line: comparison with primary RPE cultures and effect of growth medium on the alpha5 gene promoter strength. Exp Eye Res 2004; 79:157-65.
16. Wistow G, Bernstein SL, Wyatt MK, Fariss RN, Behal A, Touchman JW, Bouffard G, Smith D, Peterson K. Expressed sequence tag analysis of human RPE/choroid for the NEIBank Project: over 6000 non-redundant transcripts, novel genes and splice variants. Mol Vis 2002; 8:205-20.
17. Hollborn M, Tenckhoff S, Jahn K, Iandiev I, Biedermann B, Schnurrbusch UE, Limb GA, Reichenbach A, Wolf S, Wiedemann P, Kohen L, Bringmann A. Changes in retinal gene expression in proliferative vitreoretinopathy: glial cell expression of HB-EGF. Mol Vis 2005; 11:397-413.
18. Chowers I, Gunatilaka TL, Farkas RH, Qian J, Hackam AS, Duh E, Kageyama M, Wang C, Vora A, Campochiaro PA, Zack DJ. Identification of novel genes preferentially expressed in the retina using a custom human retina cDNA microarray. Invest Ophthalmol Vis Sci 2003; 44:3732-41.
19. Ho TC, Del Priore LV. Reattachment of cultured human retinal pigment epithelium to extracellular matrix and human Bruch's membrane. Invest Ophthalmol Vis Sci 1997; 38:1110-8.
20. Del Priore LV, Tezel TH. Reattachment rate of human retinal pigment epithelium to layers of human Bruch's membrane. Arch Ophthalmol 1998; 116:335-41.
21. Tezel TH, Del Priore LV. Repopulation of different layers of host human Bruch's membrane by retinal pigment epithelial cell grafts. Invest Ophthalmol Vis Sci 1999; 40:767-74.
22. Hubbell E, Liu WM, Mei R. Robust estimators for expression analysis. Bioinformatics 2002; 18:1585-92.
23. Liu WM, Mei R, Di X, Ryder TB, Hubbell E, Dee S, Webster TA, Harrington CA, Ho MH, Baid J, Smeekens SP. Analysis of high density expression microarrays with signed-rank call algorithms. Bioinformatics 2002; 18:1593-9.
24. Krajewski P, Bocianowski J. Statistical methods for microarray assays. J Appl Genet 2002; 43:269-78.
25. Raychaudhuri S, Sutphin PD, Chang JT, Altman RB. Basic microarray analysis: grouping and feature reduction. Trends Biotechnol 2001; 19:189-93.
26. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 1998; 95:14863-8.
27. Liu G, Loraine AE, Shigeta R, Cline M, Cheng J, Valmeekam V, Sun S, Kulp D, Siani-Rose MA. NetAffx: Affymetrix probesets and annotations. Nucleic Acids Res 2003; 31:82-6.
28. Abcouwer SF, Marjon PL, Loper RK, Vander Jagt DL. Response of VEGF expression to amino acid deprivation and inducers of endoplasmic reticulum stress. Invest Ophthalmol Vis Sci 2002; 43:2791-8.
29. Sanchez-Lopez A, Cuadros MA, Calvente R, Tassi M, Marin-Teva JL, Navascues J. Radial migration of developing microglial cells in quail retina: a confocal microscopy study. Glia 2004; 46:261-73.
30. Zagzag D, Shiff B, Jallo GI, Greco MA, Blanco C, Cohen H, Hukin J, Allen JC, Friedlander DR. Tenascin-C promotes microvascular cell migration and phosphorylation of focal adhesion kinase. Cancer Res 2002; 62:2660-8.
31. Flohe L. Selenium, selenoproteins and vision. Dev Ophthalmol 2005; 38:89-102.
32. Hecquet C, Lefevre G, Valtink M, Engelmann K, Mascarelli F. Activation and role of MAP kinase-dependent pathways in retinal pigment epithelium cells: JNK1, P38 kinase, and cell death. Invest Ophthalmol Vis Sci 2003; 44:1320-9.
33. DiSepio D, Ghosn C, Eckert RL, Deucher A, Robinson N, Duvic M, Chandraratna RA, Nagpal S. Identification and characterization of a retinoid-induced class II tumor suppressor/growth regulatory gene. Proc Natl Acad Sci U S A 1998; 95:14811-5.
34. Shi WY, Skeath JB. The Drosophila RCC1 homolog, Bj1, regulates nucleocytoplasmic transport and neural differentiation during Drosophila development. Dev Biol 2004; 270:106-21.
35. Lendeckel U, Arndt M, Wolke C, Reinhold D, Kahne T, Ansorge S. Inhibition of human leukocyte function, alanyl aminopeptidase (APN, CD13) and dipeptidylpeptidase IV (DP IV, CD26) enzymatic activities by aqueous extracts of Cistus incanus L. ssp. incanus. J Ethnopharmacol 2002; 79:221-7.
36. Lachat P, Shaw P, Gebhard S, van Belzen N, Chaubert P, Bosman FT. Expression of NDRG1, a differentiation-related gene, in human tissues. Histochem Cell Biol 2002; 118:399-408.
37. Wei W, Clarke CJ, Somers GR, Cresswell KS, Loveland KA, Trapani JA, Johnstone RW. Expression of IFI 16 in epithelial cells and lymphoid tissues. Histochem Cell Biol 2003; 119:45-54.
38. Huang LR, Hsu HC. Cloning and expression of CD24 gene in human hepatocellular carcinoma: a potential early tumor marker gene correlates with p53 mutation and tumor differentiation. Cancer Res 1995; 55:4717-21.
39. Fernandes AM, Hamburger AW, Gerwin BI. Dominance of ErbB-1 heterodimers in lung epithelial cells overexpressing ErbB-2. Both ErbB-1 and ErbB-2 contribute significantly to tumorigenicity. Am J Respir Cell Mol Biol 1999; 21:701-9.

40. Grasso AW, Wen D, Miller CM, Rhim JS, Pretlow TG, Kung HJ. ErbB kinases and NDF signaling in human prostate cancer cells. Oncogene 1997; 15:2705-16.
41. Ewart-Toland A, Briassouli P, de Koning JP, Mao JH, Yuan J, Chan F, MacCarthy-Morrogh L, Ponder BA, Nagase H, Burn J, Ball S, Almeida M, Linardopoulos S, Balmain A. Identification of Stk6/STK15 as a candidate low-penetrance tumor-susceptibility gene in mouse and human. Nat Genet 2003; 34:403-12.
42. Schmucker J, Ader M, Brockschnieder D, Brodarac A, Bartsch U, Riethmacher D. erbB3 is dispensable for oligodendrocyte development in vitro and in vivo. Glia 2003; 44:67-75.
43. Wang XF, Cui JZ, Nie W, Prasad SS, Matsubara JA. Differential gene expression of early and late passage retinal pigment epithelial cells. Exp Eye Res 2004; 79:209-21.
44. Chowers I, Kim Y, Farkas RH, Gunatilaka TL, Hackam AS, Campochiaro PA, Finnemann SC, Zack DJ. Changes in retinal pigment epithelial gene expression induced by rod outer segment uptake. Invest Ophthalmol Vis Sci 2004; 45:2098-106.
45. Rogojina AT, Orr WE, Song BK, Geisert EE Jr. Comparing the use of Affymetrix to spotted oligonucleotide microarrays using two retinal pigment epithelium cell lines. Mol Vis 2003; 9:482-96.

The print version of this article was created on 5 Jan 2006. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.