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Construction of Human Corneal Endothelial cDNA Library and Identification of Novel Active Genes

Rieko Sakai,¹ Tadoshi Kinouchi,² Shoko Kawamoto,³ M. Reza Dana,⁴ Toshiro Hamamoto,² Tadahiko Tsuru,¹ Kousaku Okubo,³ and Satoru Yamagami¹

PURPOSE. To describe genes expressed in human corneal endothelial cells and identify novel genes.

METHODS. Sixteen human donor corneas that had no history of corneal disease, infection, or intraocular surgery were used within 7 days of death. Total RNA was extracted from corneal endothelial cells with attached Descemet membranes. A 3'-directed cDNA library was constructed from mRNA by using a pUC19-based primer. These sequences were compared with each other to determine their frequency and were searched against GenBank for identification. To identify novel specific and abundant transcript genes in corneal endothelial cells, the novel genes were compared with an expressed sequence tag database, the expected sequence extended, and 5' rapid amplification of cDNA ends-polymerase chain reaction cloning performed.

RESULTS. The human corneal endothelial cDNA library showed that the most abundant transcript was prostaglandin D₂ synthase. The remaining transcript genes that were present in abundance consisted of lactate dehydrogenase-A, gene signature (GS) 3582, which is a novel gene without a known function, and matrix Gla protein. The full-length sequence of GS3582 showed similarity to genes obtained in ovary and testis.

CONCLUSIONS. A human corneal endothelial cDNA library was constructed. An expression profile of corneal endothelium provides probes to monitor physiologic and pathologic conditions of this tissue in terms of gene expression. (*Invest Ophthalmol Vis Sci.* 2002;43:1749-1756)

The corneal endothelium (CE) is a single layer of flat hexagonal cells that lies on a basement membrane, the Descemet membrane. There is no mitotic activity in the human CE after birth, which leads to a gradual decrease of the CE cell population with age as the cells migrate.¹ The CE plays a critical role in controlling stromal hydration, including the barrier and pump function of the aqueous humor and ion transport. In addition, the cells have metabolic activity as

indicated by the presence of mitochondria, endoplasmic reticulum, Golgi apparatus, and free ribosomes.² Gene analysis of CE could provide molecular clues for essential functions of the CE, including the maintenance of corneal transparency.

In the human eye, gene expression profiles have been investigated for the corneal epithelium,³ conjunctival epithelium,⁴ trabecular meshwork,⁵ and retina.⁶ Much of the corneal stroma, the CE, and iridocorneal angle (trabecular meshwork) are derived from neural crest, whereas the corneal and conjunctival epithelium and sensory retina are derived from surface ectoderm and neural ectoderm, respectively, suggesting that the genes expressed in the CE may be distinct from those in the epithelium of the cornea or conjunctiva. A cDNA library from cultured rabbit CE⁷ has been characterized. However, human CE (HCE) does not regenerate in vivo, reflecting an essential difference from the regenerative capacity of rabbit CE.⁸ Therefore, gene expression profiles of noncultured HCE could provide important details on the in vivo function of HCE.

Unique gene expression analyses based on 3'-directed cDNA contain a short sequence of mRNA polyA+ tails through the nearest *Mbo*I site. The information is called a gene signature (GS) and consistently represents the mRNA population in a given tissue.^{9,10} The *Mbo*I site is used, because GATC is the most uniformly distributed recognized sequence for commercially available four-base cutters in human gene sequences. By comparing the expression profile for a particular tissue with profiles from other cells, genes can be categorized into those that are active in many other source tissues.

In this study, we describe the gene expression profile of the HCE by using the GS system and we identify the full-length sequence of a novel gene. These results may provide important candidate genes for the study and understanding of the physiological and metabolic functions of the HCE.

MATERIALS AND METHODS

Preparation of HCE

Sixteen human corneas that had no history of corneal disease, infection, or intraocular operation, were provided from the American Eye Bank of Florida. The average age of these corneas was 59.9 ± 5.9 years (age range, 49-70). All corneas were kept in storage medium (Optisol GS; Chiron Vision, Irvine, CA) at 4°C and were used within 7 days of death. This storage medium consists of a hybrid of K-Sol (Chiron Vision) and Dexol (Chiron Vision) containing 2.5% chondroitin sulfate, 1% dextran, vitamins, precursors of adenosine triphosphate (adenosine, inosine, and adenine), gentamicin, and streptomycin.^{11,12} After excising the clear cornea with scissors, the CE and Descemet membrane were peeled away in a sheet from the periphery to the center with fine forceps. Based on our investigations, correlating histologic findings with our microsurgical procedures, posterior stroma adhering to the Descemet membrane leads to considerable resistance in excising the CE-Descemet complex from the remainder of the cornea. To avoid the inclusion of posterior stromal tissue, only strips of CE-Descemet tissue that were excised smoothly from the stroma, without residual stroma, were used in the experiments. The removed CE was immediately homogenized in RNA isolation agent (RNAzol B; Tel-Test, Inc., Friendswood, TX), and stored at -70°C until use.

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Construction of a 3'-Directed cDNA Library

Total RNA was extracted from the homogenized solution. PolyA+ RNA was prepared by using an mRNA purification kit (QuickPrep; Amersham Pharmacia Biotech, Tokyo, Japan) according to the manufacturer's instructions. A 3'-directed cDNA library was constructed using polyA+ RNA, as described previously.¹¹ Briefly, pUC19-based vector primer was used for cDNA synthesis, and the 5' side of double-stranded was trimmed off with *Mbo*I (GATC) to decrease the transforming efficiency bias caused by size difference.¹² Cohesive termini were created at the other end of the vector molecules by cleaving with *Bam*H1 and were self-ligated. By transforming them into *Escherichia coli* DH5 (Toyobo, Osaka, Japan) and using one tenth of the mixture, we obtained approximately 2.5×10^5 independent transformants. With this shortening of the cDNAs using *Mbo*I, we could diminish the transforming efficiency bias caused by size differences.¹⁰

Sequence and Data Analysis

The transformed colonies were selected randomly and lysed. The cDNA moiety was then amplified by polymerase chain reaction (PCR), using a primer pair that flanked the insert (5'-GTTTCCCAGTCAC-GACGTTG-3'; 5'-ACCATGATTACGCCAGCTTG-3'), as described elsewhere.¹² The PCR products were subjected to a cycle sequencing reaction, using dye primer M13, and analyzed by autosequencer (model 373A; PE Biosystems, Foster City, CA). Inserted sequences shorter than 20 bp were eliminated from analysis because of inadequate length to discriminate among approximately 10^5 transcripts at their 3' termini. Inserts that had no unique sequences or had more than 5% ambiguous nucleotides were also eliminated. Sequences were compared to each other using the FastA program,¹³ and identical sequences were grouped together as a single gene species. Sequences with more than 95% identical bases throughout the query sequences were regarded as identical. For recurring sequences that appeared more than twice, homologies were searched in a daily updated expressed sequence tag database (dbEST) using the Basic Local Alignment Tool (BLAST) of the National Center for Biotechnology Information (NCBI, Bethesda, MD; available in the public domain at <http://www.ncbi.nlm.nih.gov/BLAST>).

Full-Length Sequencing

The novel and abundant transcript gene, GS3582, in our established HCE cDNA library, corresponded to the daily updated dbEST and extended the expected sequence. 5'-Rapid amplification of cDNA ends (RACE)-PCR cloning was performed using a kit (Sure-RACE; Origene Technologies, Inc., Rockville, MD) that allows isolation of the 5' sequence of the target transcript. The RACE panels in the kit consist of double-stranded cDNA from 24 individual human tissues arrayed in a multiwell plate and provides two contiguous adapter-specific primers at the 5' end. Outer primer for first-round PCR, gene-specific primer (GSP)-1: 5'-TTTCCGCAACATTCTCCTTTT-3') and inner primer for second-round (nested) PCR, GSP2: 5'-TTCTGTGTTGGCTTGGT-3') were designed from the expected sequence.

The RACE cDNAs were amplified with DNA polymerase (KOD-Plus; Toyobo). After incubation at 94°C for 3 minutes, the first round of PCR was performed with 5 cycles of 94°C for 3 seconds, 63°C for 30 seconds, and 72°C for 3 minutes and then 15 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 6 minutes, followed by 72°C for 6 minutes in a PCR thermal cycler (model MP; Takara, Kyoto, Japan). After incubation at 94°C for 3 minutes, the second round of PCR was performed with 35 cycles of 94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 3 minutes, followed by 72°C for 6 minutes. Among human cDNAs in different tissues, PCR products 300 to 400 bp in length were circularized and subcloned into a vector (pBluescript; Stratagene, La Jolla, CA). Randomly selected plasmids were amplified and purified into DNA with a kit (Plasmid Midi; Qiagen, Hilden, Germany). The products were amplified with T7 promoter primer (T7 HT Primer; Toyobo) with 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes after incubation at 96°C for 30

TABLE 1. Distribution of Gene Signatures among Different Occurrence Groups

Frequencies	Species		Clones	
	Known	Unknown	Known	Unknown
≥10	35	1	522	21
5-9	45	4	289	23
3-4	76	20	251	64
2	167	47	334	94
1	623	442	623	442
Subtotal	946	514	2019	644
Total		1460		2663

A total of 1460 gene signatures representing different genes are grouped according to the frequencies of appearance. Known or unknown means whether the sequences of the genes are identified in GenBank or not.

seconds. Amplified products were sequenced with the autosequencer (ABI Prism 310 Genetic Analyzer; PE Biosystems). The resultant sequence was extended to the expected sequence, and the analysis of the total GS3582 sequence was concluded.

RESULTS

An Expression Profile of Active Genes in Human Corneal Endothelium

The frequency distribution for the GSs is listed in Table 1. We used 5125 transformants randomly for sequence and obtained 1460 independent GS species comprising 2663 clones by eliminating mitochondrially coded sequences, ribosomal RNAs, repetitive sequences, and low-quality sequences having more than 5% ambiguous bases. Among them, 946 (64.8%) species represented by 2019 clones, were identified in GenBank (GenBank is provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD, and is available at <http://www.ncbi.nlm.nih.gov/Genbank>), and the remaining 514 (35.2%) species, represented by 644 clones, were novel genes. Three hundred ninety-five GSs appeared recurrently, representing genes that are highly or moderately active in the human CE. One thousand sixty-five GSs appeared only once. All the GSs collected from the HCE were compared with sequences obtained previously from other tissues. The table listing all the GS found in the corneal tissue is referred to as a gene expression profile of corneal endothelium. It can be obtained through our BodyMap Server (Tokyo University, Tokyo, Japan; available at <http://bodymap.ims.u-tokyo.ac.jp>).

Gene Expression Profile of Human Corneal Endothelium

Part of the expression profile listing active genes in the order of abundance of transcript genes is shown in Table 2A. The most abundant transcript was prostaglandin (PG) D₂ synthase ($n = 68$). The following transcript genes consisted of lactate dehydrogenase-A ($n = 28$); GS3582 ($n = 21$), a novel gene; and matrix Gla protein ($n = 17$). To reveal the gene expression specific to the HCE, the number of GSs detected in HCE were compared with that of GSs in other tissues obtained previously in our BodyMap. The gene ratio in HCE was calculated according to the following formula: gene ratio = detected gene number in the HCE divided by the total gene number of other tissues including HCE genes in BodyMap. Genes expressed with high frequency in the HCE compared with those in other tissues are listed in Table 2B. Damage-specific DNA-binding protein 2 (48 kDa; $n = 5$) was found only on the CE among five GSs. The following genes with high rates of expression in the

TABLE 2. Gene Expression Profiles of Corneal Endothelium

A. In Order of Frequency of Appearance				
GS	CE	Accession Number	Definition	
2851	68	M98539	Prostaglandin D ₂ synthase	
463	28	X02152	Lactate dehydrogenase-A (LDH-A)	
418	21	U09953	Ribosomal protein L9	
3582	21		Unknown*	
285	19	X89401	Ribosomal protein L21	
336	19	Z12962	Ribosomal protein L41	
2712	17	X53331	Matrix Gla protein	
543	16	X56932	23 kDa highly basic protein	
818	16	U14966	Ribosomal protein L5	
290	15	X66699	Ribosomal protein L37a	
304	15	M26880	Ubiquitin	
375	15	L38941	Ribosomal protein L34	
14518	15	M29458	Carbonic anhydrase III	
437	14	L16558	Ribosomal protein L7	
212	12	M25639	Migration inhibitory factor (MIF)	
273	12	X16064	Translationally controlled tumor protein	
356	12	X53777	Ribosomal protein L23	
363	12	D14696	HepG2 identical sequence	
422	12	M10036	Triosephosphate isomerase	
444	12	M81757	Ribosomal protein S19	
512	12	M58458	Ribosomal protein S4	
861	12	M31520	Ribosomal protein S24	
335	11	X52138	Ribosomal subunit (L7a)	
447	11	X69391	Ribosomal protein L6	
743	11	U14973	Ribosomal protein S29	
807	11	X69392	Ribosomal protein L26	
865	11	Z11531	Elongation factor-1- γ	
1617	11	M14061	U3	
1919	11	J02763	Calcyclin	
2380	11	NM_015965	CGI-39 protein	
19	10	X16869	Elongation factor 1- α	
797	10	J02984	Insulinoma rig-analogue encoding DNA-binding protein	
1905	10	BC001031	Muscle-specific gene, clone MGC:1542	
1953	10	NM_005004	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8	
2767	10	U37690	RNA polymerase II subunit (hsRPB10)	
3663	10	S41243	Amyloid protein precursor	
102	9	X64707	BBC1	
292	9	M60854	Ribosomal protein S16	
293	9	U14968	Ribosomal protein L27a	
2013	9	Y14551	DIF-2 protein	
6139	9	AB007510	PRP8 protein	
262	8	M17885	Acidic ribosomal phosphoprotein P0	
456	8	NM_006886	ATP synthase	
644	8	U12465	Ribosomal protein L35	
839	8	L20216	<i>Cercopithecus aethiops</i> UV-damaged DNA-binding protein 127-kDa subunit	
934	8	X58139	Cox VIb	
5837	8	AF069073	P8 protein	
305	7	U14969	Ribosomal protein L28	
315	7	L06498	Ribosomal protein S20	
500	7	D14530	Ribosomal protein S28	
521	7	X69181	Ribosomal protein L31	
716	7	X56999	Uba52	
759	7	M13932	Ribosomal protein S17	
2394	7	NM_006476	ATP synthase, H ⁺ transporting, mitochondrial F1F0, subunit γ (ATP5JG)	
2793	7		Unknown	
B. In the Order of Rate of Appearance				
GS	CE	CE/total	Accession Number	Definition
21799	5	1.00	NM_006476	Damage-specific DNA binding protein 2 (DDB2)
3582	21	0.75		Unknown*
3478	4	0.67	AK021780	<i>Homo sapiens</i> mRNA for KIAA0585 protein
14518	15	0.52	M29458	Human carbonic anhydrase III gene
2851	68	0.48	M98539	Prostaglandin D ₂ synthase
8870	6	0.46	AF056087	Secreted frizzled related protein
1617	11	0.46	M14061	U3
2712	17	0.43	X53331	Matrix Gla protein

(continues)

TABLE 2 (continued). Gene Expression Profiles of Corneal Endothelium

B. In the Order of Rate of Appearance				
GS	CE	CE/total	Accession Number	Definition
5837	8	0.42	AF069073	P8 protein
6501	5	0.38	NM_014306	Similar to <i>Caenorhabditis elegans</i> hypothetical 55.2 kDa protein F16A11.2 (DJ149A16.6)
6139	9	0.36	AB007510	PRP8 protein
2380	11	0.33	NM_015965	CGI-39 protein
84	5	0.28	K03195	Glucose transporter
478	4	0.25	NM_016558	SCAN-related protein RAZ1 (SDP1)
2013	9	0.24	Y14551	DIF-2 protein
840	4	0.24	Z49099	Spermine synthase
1354	6	0.23	S77356	Oligomycin sensitivity conferral protein homologue
839	8	0.23	L20216	<i>Cercopithecus aethiops</i> UV-damaged DNA-binding protein 127-kDa subunit
1489	5	0.23		Unknown

* GS3582 has been submitted to GenBank (Accession number: Af484960).

The gene signatures (GS) that appeared equal to or more than 7 times in corneal endothelium are shown in A. Part of expression profile of corneal endothelium in the order of the gene ratio in HCE as compared with that in other tissues. Gene ratio in HCE was calculated according to the following formula: gene ratio = detected gene number in the HCE ÷ total gene number of other tissues including HCE gene in the Bodymap. The gene signatures (GS) that appeared equal to or more than 4 times in corneal endothelium are included in B. GS, gene signature; CE, corneal endothelium; CE/total, the ratio of GS number in corneal endothelium, divided by total GS number detected in other tissues including CE of Bodymap.

CE were the novel gene, GS3582 (75% of GSs listed in the BodyMap are found in CE), highly similar to *Homo sapiens* mRNA for KIAA0585 protein (67%), and human carbonic anhydrase 3 (52%).

We categorized the genes identified in GenBank according to the function and subcellular localization. Four hundred fourteen clones (20.5%) were related to ribosomal component and translational factors, 243 clones (12%) to signal transduction, 229 (11.3%) to cytoplasmic proteins, 210 (10.4%) to nuclear proteins and transcriptional factors, 163 (7.7%) to mitochondrial protein, and 118 (7.3%) to membrane protein. Among the 945 GSs identical with known genes, those encoding signal transduction, cytoplasmic proteins, membrane proteins, secretory and extracellular proteins, and cytoskeleton proteins are listed in Table 3.

Isolation of a Full-Length cDNA for GS3582

We chose GS3582 to investigate the HCE-specific gene, because GS3582 is an transcript gene that is abundant in the HCE with a higher proportion rate than transcript genes in other tissues in our BodyMap. To verify the distribution of GS3582 in human organs, Northern blot analysis was performed with a kit (Human 12-Lane MTN Blot; Clontech Laboratories, Palo, Alto, CA). However, GS3582 was not detected in a panel of human mRNA (data not shown). Therefore, the products of 5' RACE-PCR cloning were examined in the human cDNA in the different human tissues. PCR products of 300 to 400 bp in length were identified in the pituitary, the prostate, the testis, and the ovary (Fig. 1).

The cDNA sequence and gene structure of GS3582 are shown in Figure 2. This sequence contains an open reading frame of 240 amino acids, with a molecular weight estimated at 25.4 kDa. The human genome database shows that GS3582 locates to human chromosome 4q28 (the nearest gene is nicotinamide-adenine dinucleotide [NADH] dehydrogenase [ubiquinone], or *NDUFC1*). Each exon of the gene is scattered over 14 kbp (Fig. 1B) and is completely consistent with a sequence in the database. The sequence is rich in the acidic amino acids (glutamic and aspartic acids), which are present at a ratio of 19% (46/240), and the theoretical isoelectric point is 4.24. According to protein motif analysis (PROSITE, Swiss Institute of Bioinformatics, Geneva, Switzerland, <http://expasy.nhri.org.tw/prosite/>; BLOCKS, the Fred Hutchinson Cancer Research Center, Seattle, WA, <http://www.blocks.fhrc.org/>;

ProDom, The Protein Domain Database, Toulouse, France; <http://prodes.toulouse.inra.fr/prodom/doc/prodom.html>; PRINTS, Protein Fingerprint Database, University of Manchester Bioinformatics Education and Research, Manchester, UK, <http://bioinf.man.ac.uk/dbbrowser/PRINTS/>; and Pfam, The Sanger Centre, Hinxton Hall, UK; <http://www.sanger.ac.uk/Pfam/>), GS3582 does not possess any characteristic sequences such as a signal peptide or other localization motifs.

The N-terminal 238-amino-acid sequence of GS3582 is identical with the human ovary-specific acidic protein,¹⁴ consisting of 268 amino acids, according to the BLAST program. Two genes with high homology in human GS3582 were found in the rabbit corneal endothelial cDNA library and RIKEN (The Institute of Physical and Chemical Research, Tokyo, Japan, <http://www.riken.go.jp/>) full-length enriched library established from adult mouse testis cDNA. The homologue of cultured rabbit corneal endothelium codes the 3' untranslated region and exhibits a 66.5% homology for human GS3582. The mouse testis has a large number of aspartic and glutamic acid residues (61 of a total 283) and a protein, translated from the mouse testis gene, possesses significant homology with the human GS3582 at the N-terminal (amino acid positions 1–60) sequence. In the mouse testis gene, two large insertion sequences exist in the C-terminal region and there are three tandem repeats, Glu-Gly-Ala-Asp-Thr-Ser-Gln, in the first insertion sequence.

DISCUSSION

In the present study, we sought to construct a human corneal endothelial cDNA library by evaluating gene expression in 16 human corneas with no history of disease. Because the donor corneas were stored in storage medium (Optisol GS; Chiron) and the HCE samples were acquired from the donors within 7 days of death, it is possible that the data may not precisely reflect the in vivo expression profile due to the possible effect of the storage medium on select genes after death. However, to the best of our knowledge, storage in Optisol GS does not affect the G₁ phase of the cell cycle in the human corneal endothelium.¹⁵ Moreover, similarly (or even significantly longer) stored tissue is used routinely in clinical transplantation, and the CE functions well after grafting leading to corneal transparency. These results suggest that although the gene

TABLE 3. Gene Expression Profiles Categorized by Function and Subcellular Localization

GS	CN	Accession Number	Definition
Signal transduction			
212	12	M25639	Migration inhibitory factor (MIF)
273	12	X16064	Translationally controlled tumor protein
1919	11	J02763	Calcyclin
2380	11	NM_015965	CGI-39 protein (LOC51079)
2013	9	Y14551	DIF-2 protein
5837	8	AF069073	P8 protein
2821	6	X68277	CL 100 protein tyrosine phosphatase
374	5	AJ005259	EDF-1 protein
943	5	S75476	Phosphoglycerate kinase 1 (PGK1)
1431	5	X51346	Jun-D protein
904	4	NM_015710	Glioma tumor suppressor candidate region gene 2 (GLTSCR2)
177	3	L19739	Metallopanstimulin (MPS1)
958	3	NM_007065	CDC37
1983	3	S44881	β -Galactoside binding protein
2988	3	S57501	Protein phosphatase type 1 catalytic subunit
3529	3	D78014	Dihydropyrimidinase related protein-3
3888	3	U34343	13kD differentiation-associated protein
6649	3	AL022729	<i>Homo sapiens</i> RAY1
7106	3	D42123	ESP1/CRP2
14955	3	U78580	Type I phosphatidylinositol-4-phosphate 5-kinase β (STM7)
17076	3	L19314	HRY gene
Cytoplasmic proteins			
2851	68	M98539	Prostaglandin D ₂ synthase
463	28	X02152	Lactate dehydrogenase-A (LDH-A)
304	15	M26880	Ubiquitin
14518	15	M29458	Carbonic anhydrase III
716	7	X56999	Uba52
600	5	D78134	CIRP
134	3	J04988	90 kDa heat-shock protein
1706	3	M80563	CAPL protein
2313	3	X61971	Macropain subunit delta
5108	3	Z23090	28-kDa heat-shock protein
Membrane proteins			
3663	10	S41243	Amyloid protein precursor
182	6	M84349	Transmembrane protein (CD59)
84	5	K03195	Glucose transporter
3775	5	L11373	Protocadherin 43
279	3	AF298897	Amino acid transporter system A (ATA2)
937	3	X64881	HLA-E heavy chain
1796	3	L20859	Leukemia virus receptor 1 (GLVR1)
2514	3	U70660	Copper transport protein HAH1
Secretory and extracellular proteins			
2712	17	X53331	Matrix Gla protein
3200	6	J03040	SPARC/osteonectin
8870	6	AF056087	Secreted frizzled related protein
150	5	M27891	Cystatin C (CST3)
2186	3	M74816	Apolipoprotein J
19008	3	X15005	Laminin-binding protein (nem/lcHD4)
Cytoskelton proteins			
155	4	M17733	Thymosin β -4
3013	4	M37984	Slow-twitch skeletal muscle/cardiac muscle troponin C
258	3	X04588	2.5-kb Cytoskeletal tropomyosin TM30 (nm)

In the 2019 clones identified in GenBank, those categorized for signal transduction, cytoplasmic proteins, membrane proteins, secretory and extracellular proteins, and cytoskeleton proteins are listed.

expression profile of eye bank tissue may not be completely identical, it is fundamentally similar to that of the in vivo cornea.

The CE is metabolically active and therefore requires nutrients for its function. Glycogen and glucose from the aqueous humor are the main energy sources for endothelial cells. Glucose is catabolized through aerobic pathways, including the tricarboxylic acid cycle and hexose monophosphate shunt, and anaerobic pathways. However, whether the CE has the capacity to store glycogen is not known.¹ The active genes such as lactate dehydrogenase A ($n = 28$), NADH dehydrogenase ($n = 10$), and adenosine triphosphatase (ATPase, $n = 8$)

express abundantly, suggest dynamic glucose metabolism in the corneal endothelium. The control of corneal stromal hydration sustained by pump and barrier function in the corneal endothelium is essential for transparency. The abundant adenosine triphosphatase (ATPase) and carbonic anhydrase gene ($n = 15$) may explain the pump function in relation to Na-K, ATPase.

Physiologic extracellular matrix calcification is restricted to bones, teeth, and the hypertrophic zone of growth plate cartilage. Matrix Gla ($n = 17$) is the protein associated with protection against calcification in soft tissues.¹⁶ Nonsense mutations in the human matrix Gla protein gene cause Keutel

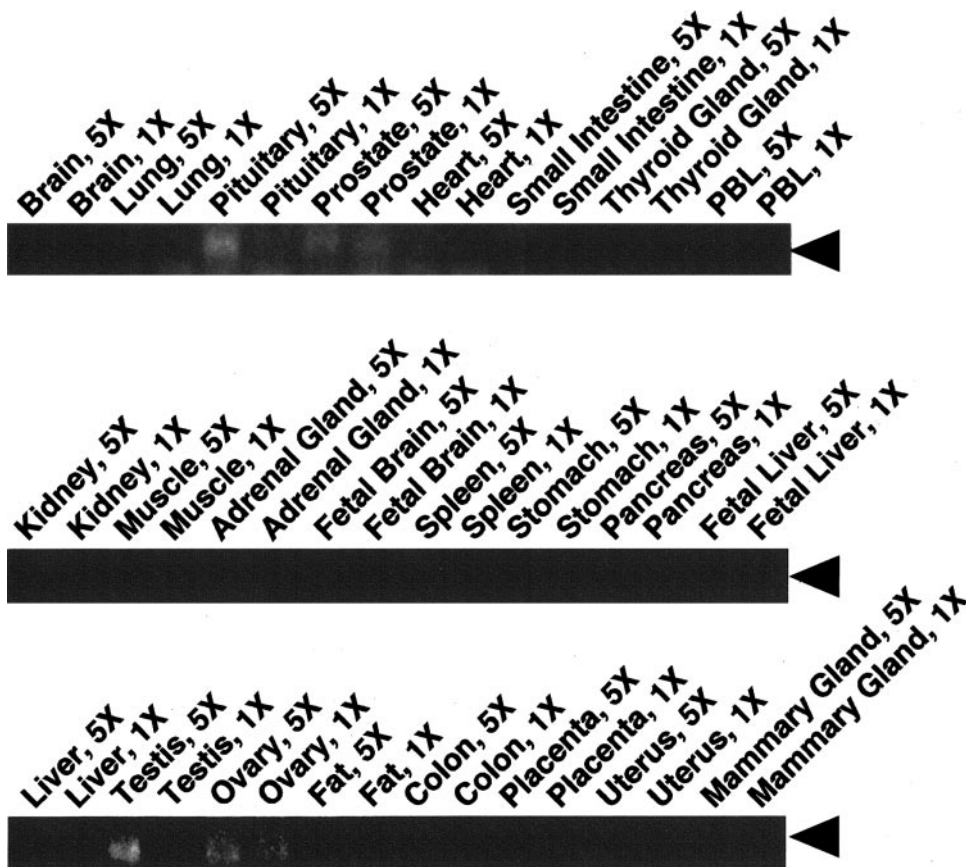


FIGURE 1. The products of 5' RACE-PCR cloning are shown in the human cDNA in different tissues. PCR products are identified in pituitary, prostate, testis, and ovary. A 5× sample is five times the concentration of 1×.

syndrome, a rare autosomal recessive disorder characterized by diffuse cartilage calcification.¹⁷ In mice, deletion of matrix Gla protein causes cartilage calcification and arterial calcification.¹⁶ In the CE, calcium ions in the aqueous humor are necessary for the maintenance of the barrier function of the CE. The existence of matrix Gla protein gene in the HCE suggests that the protein may metabolize calcium ions that maintain barrier function and control stromal hydration while protecting calcium deposit in the cornea. SPARC/osteonectin ($n = 6$) in secretory and extracellular proteins also could be associated with the barrier function of the CE.¹⁸

PGD is a neuromodulator that participates in the sleep-wake cycle, thermoregulation, and odor responses.¹⁹ There are two enzymes that catalyze the conversion of PGH₂ into PGD₂: hematopoietic PGD synthase and lipocalin-type PGD synthase.^{20,21} Not only brain, but also the epididymis and testis express a high amount of lipocalin-type PGD synthase protein.²²⁻²⁴ In the eye, lipocalin-type PGD synthase is synthesized within the epithelial cells of the iris-ciliary body and pigment epithelium and is secreted into the aqueous and vitreous humors, respectively.²⁵⁻²⁷ The gene transcript of PGD₂ synthase ($n = 68$) is the most abundant transcript gene in the present study, suggesting that CE covering the anterior chamber is also the major source of PGD₂ synthase secretion and that PGD₂ may be involved in the regulation of intraocular pressure.²⁸

It is of interest that β -amyloid protein precursor gene (APP, $n = 10$) is detected in the CE. Disruption of the normal function of the APP is known as a leading cause of Alzheimer disease.²⁹ After a wound to the cornea, APP is upregulated in the basal cells of the corneal epithelium that is actively migrating,^{30,31} but not in the normal human corneal epithelium,³ implying a role for mediating epithelial migration during reepithelialization. Because *in vivo* the HCE does not show any regenerative ability, it heals only by migration, suggesting a

critical role for APP in CE migration. Further study is needed to reveal the exact mechanism that mediates migration in the HCE.

GSS found only in corneal endothelium included damage-specific DNA-binding protein 2 (DDB2; $n = 5$). Ultraviolet (UV) radiation-damaged DNA-binding activity requires the expression of two subunits, p127, DDB1 ($n = 8$), and p48, DDB2,³² which are detected in the HCE library, but not in corneal epithelium.³ Clinically, xeroderma pigmentosum group E cells, induced by a nonsense mutation of DDB2,³³ are deficient in global genomic repair.³⁴ Moreover, decreased cell density and increased coefficient of variation in cell area have been reported in patients with xeroderma pigmentosum.³⁵ Nonregenerative HCE, different from regenerative corneal epithelium, may be necessary to prepare an efficient genomic system for repair of DNA damaged by UV radiation, with the aid of DDB2 protein.

As for the previously established cDNA library of anterior segment tissues of the eye, our library of noncultured HCE is not entirely compatible with that of cultured rabbit CE.⁷ This may be due to not only species differences, but also to the samples' originating from cultured versus noncultured cells. There are few common gene expressions among the cDNA libraries derived from HCE and human corneal epithelium.³ Perhaps this is not surprising, given the differences in embryologic origins between these two cell types, the former derived from neural crest and the latter from surface ectoderm. In contrast, abundant genes, including lactate dehydrogenase and matrix Gla protein, in the CE coincide well with those in the human trabecular meshwork⁵ cDNA library, which also has a neural crest origin. Moreover, cDNA libraries of human iris-ciliary body and CE have similar gene expression profiles in our BodyMap (data not shown). These results suggest that ocular tissues originating from the neural crest surrounding the ante-

A

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10      20      30      40      50      60
tcagcctctctgtggaagggcagcctgcgctgggtaccgaggtgctgcgcgggcca

70      80      90      100     110     120
gcgggcgcgatgtatctccgagggcggtctccaagactctggcgctgectgaggcg
M Y L R R A V S K T L A L P L R A

130     140     150     160     170     180
cccccaaccccgcgctcggaaggaagcctctgcgccggatgcatcctaacaga
P P N P A P L G K D A S L R R M S S N R

190     200     210     220     230     240
ttcctggatcatctggatcaaatatgatttattatctgggttagcgctcacagtcag
F P G S S G S N M I Y Y L V V G V T V S

250     260     270     280     290     300
gctggtggatattatgcttacaagacagtcacatcagaccaagccaacacacogaacat
A G G Y Y A Y K T V T S D Q A K H T E H

310     320     330     340     350     360
aaaacaatttgaagaaaaaacaagcagagatacatccatttcaaggtgaaaaggag
K T N L K E K T K A E I H P F Q G E K E

370     380     390     400     410     420
aatgttgcggaaactgagaagcaagttcagaagcccagaagaacttatagtggaagct
N V A E T E K A S S E A P E E L I V E A

430     440     450     460     470     480
gaggtgtagatgctgaagaaagtcacagtgctacagttgtggtcataaaaggagcatct
E V V D A E E S P S A T V V V I K E A S

490     500     510     520     530     540
gcctgtccaggtcagtgaggctgctccggagaccaagcagtcagtgctgaaaccggg
A C P G H V E A A P E T T A V S A E T G

550     560     570     580     590     600
ccagaggtcacagatgcagcggcgagggaaccacggaagtaaacctgaaacaacccca
P E V T D A A A R E T T E V N P E T T P

610     620     630     640     650     660
gaggttacaaatgctgcccctggatgaagctgtcaccatcgataatgataaagatacaaca
E V T N A A L D E A V T I D N D K D T T

670     680     690     700     710     720
aagaacgaaaccttgatgaatgctgaactagaagaagaaaattctccagctgagtcac
K N E T S D E Y A E L E E E N S P A E S

730     740     750     760     770     780
gagtcctctgctggagatgatttccaggggaagccagtggttgctctgaggctgcttcg
E S S A G D D L Q E E A S V G S E A A S

790     800     810     820     830     840
gctcaaggctaatctccagccggtagacatttcagcaacaatgccatagggtgctgtag
A Q G *

850     860     870     880     890     900
aagtgtctttgtgttttagtacaacttagtttaaaaaaaaaaagacttattttctagaaa

910     920     930     940     950     960
acgttaatgggtcttgaagatttttggcatccactgatagatttttagactgagagactt

970     980     990     1000    1010    1020
gagattgtacatttttactactcttctactgtctgagatcagaatatgacattgacgta

1030    1040    1050    1060    1070    1080
aagagcttaaatagtttcatcttgggttttttttctagacacttttctttatcccagct

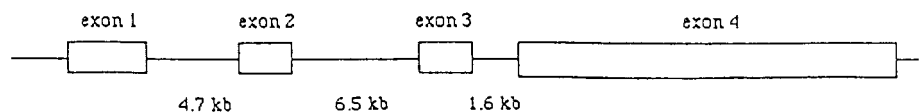
1090    1100    1110    1120    1130    1140
catctctgaatttcattttatataaagaattgagctatccatacaacctgtattttac

1150    1160    1170    1180    1190    1200
tttaatttcaactatgcttccagcttactttattgtatgatgtaggtctaaatotagt

1210    1220    1230
ttgagtcaaataaattagaaaagctttaa
    
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FIGURE 2. (A) The sequence of GS3582. The open reading frame was analyzed and translated into the amino acid sequence. This sequence contains 240 amino acids, and the molecular weight is estimated at 25.4 kDa. GS3582 locates on human chromosome 4 and completely matches with the sequence of the human genome database. (B) Gene structure of GS3582.

B



rior chamber can provide common proteins for the maintenance of aqueous humor physiology.

We chose GS3582 ($n = 21$), a novel gene with a high rate of expression in the HCE, and isolated a full-length cDNA. As shown in Figure 2, GS3582 does not possess a signal peptide at N termini, indicating that GS3582 is not a secretory protein and could be classified in the intracellular organelle or cytoplasm. By using the Web server at NCBI, significant homology (E value of $3e^{-98}$) was observed between GS3582 and the ovary-specific acidic protein (GenBank accession number, AF329088).¹⁴ The ovary-specific acidic protein was so named because of the restricted expression to the ovary by Northern blot analysis of the other systemic tissues not including human corneas. Moreover, a similar full-length sequence was also obtained in the mouse testis (accession number: AK006339 in NCBI). These results suggest apparent polymorphisms for GS3582. GS3582 is found in the human corneal epithelium, but not retina or iris in our BodyMap, and in the human cDNA of pituitary, prostate, testis, and ovary. Further studies should be conducted to reveal possible biological functions shared among these tissues and cornea. It is of interest that the ovary, testis, and anterior chamber all represent immune-privileged tissues. As such, the sharing of specific gene products among these tissues may provide some insight into similarities they share in terms of immunity.³⁶

In summary, we have constructed a cDNA library of the HCE based on analysis of 2663 clones and have reported a full-length sequence of a novel gene showing the similarity to genes obtained in the ovary and the testis. Our cDNA library of HCE could provide new insights into the physiology of HCE.

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