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Cell and tissue specific expression of human Krüppel-like transcription factors in human ocular surface

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Purpose: The ocular surface, composed of the conjunctiva and the cornea, is essential for vision. Its integrity depends on numerous molecular and cellular processes such as proliferation, differentiation, apoptosis, adhesion, and extracellular matrix homeostasis, whose deregulation can induce ophthalmological pathologies. The Krüppel-like transcription factors (KLFs) family is made up of 15 C_2H_2 zinc-finger proteins involved in vertebrate development and able to control cell proliferation, growth, and differentiation. In order to better define their respective roles in the human ocular surface, we decided to determine their pattern of expression in ocular tissues. We then focused on the expression of KLF4 and some of its target genes to establish KLF4's biological activities in human ocular surface.

Methods: Firstly, total mRNA was extracted from human total cornea, conjunctiva, corneal epithelial cells (primary culture and established cell line), corneal keratocytes (primary culture), corneal endothelial cells (established cell line), and conjunctival epithelial cells (established cell line) and submitted to RT-PCR experiments in order to determine the expression patterns of the different KLFs. Secondly, KLF4 protein localization was visualized by immunofluorescence assays at tissue and cell levels. Finally, KLF4 target genes (endoglin, ornithine decarboxylase) mRNA expression levels were determined by semi-quantitative RT-PCR, after KLF4 transient transfection in human corneal epithelium (HCE) cells.

Results: We detected the presence of twelve transcripts of KLFs in the cornea (KLF2, KLF3, KLF4, KLF5, KLF6, KLF7, KLF8, KLF10, KLF11, KLF12, KLF13, and KLF16) and eight in the conjunctiva (KLF2, KLF3, KLF4, KLF6, KLF7, KLF10, KLF11, and KLF12). Under our conditions, the transcripts encoding KLF1 and KLF9 were never detected. Specific expression patterns of each KLF were also determined for the major cellular components of the human cornea and conjunctiva. KLF4 immunolocalization assays indicated its presence in both the cytoplasmic and nuclear compartments of conjunctival and corneal cells. KLF4 transient overexpression in HCE cells down regulated both endoglin and ODC mRNA levels.

Conclusions: For the first time, we established the presence of a KLF network in the human ocular surface and illustrated the conservation of KLF4's biological properties in a corneal derived epithelial cell line.

The ocular surface, composed of the conjunctiva and the cornea, is the first anatomic structure involved in visual perception. Its normal development, and homeostasis are essential for vision, and rely on numerous cellular processes, such as epithelial and endothelial cell growth, proliferation, differentiation, and apoptosis [1]. Central to the control of these processes are DNA binding transcriptional regulators. Amongst them is the evolutionary conserved family of C_2H_2 zinc-finger Krüppel-like factors (KLFs) [2-7]. KLFs are uniquely expressed in a large variety of tissues, bind to similar "GT box or CACCC element" sites on DNA, and function as transcriptional activators or, repressors, or both [2-4]. Fifteen KLF coding genes have been thus far identified in the human, and mouse genomes, and segregated into four phylogenetically distinct groups based on structural considerations [4]. KLFs have been shown to regulate growth, proliferation, and differentiation of distinct cell lineages by binding to similar DNA elements in the promoters of house keeping, and tissue specific genes [2-4]. For example, KLF4 has been shown to stimulate p21 accumulation and growth arrest in cell cultures and to direct terminal differentiation of dermal and intestinal epithelia in mice [8-10]. We previously showed that Luna, the invertebrate progenitor of the mammalian KLF6/KLF7 transcription factors, was essential for Drosophila development and targeted perturbation of its function in the eye indicated its involvement in terminal differentiation rather than cell specification [7]. We also demonstrated that KLF6 is implicated in corneal physiology and integrity by its ability to regulate the keratin K12 expression, a structural component of the corneal epithelium [11]. Recently, KLF4 was described to be expressed during murine eye development (Personal communication, H. Sakai, May 2004), and in postnatal mouse cornea at an abundant level [12]. All together these data strongly support the involvement of the KLFs in eye development and homeostasis.

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In order to better define the KLFs' respective functions in the human ocular surface, we decided, firstly, to determine their pattern of expression by anatomic region. We then focused on the expression of the protein KLF4 and verified its biological ability to regulate two previously described target genes: endoglin and ornithine decarboxylase. Our results strongly suggest that the KLFs play a role in human ocular surface physiology and illustrate the conservation of KLF4's biological role among different epithelia.

METHODS

Collection of human tissues: Pieces of normal human bulbar conjunctiva were removed from 10 patients during ocular surgery, after informed consent, according to the tenets of the Declaration of Helsinki. The research was approved by the institutional human experimentation committee (Regional Ethics Committee). Five normal human corneas unsuitable for graft for serological reasons were provided by the regional eye bank. All tissues were rinsed using cold sterile phosphate buffered saline (PBS), embedded with Tissue-Teck OCT (Sakura, Zoeterwounde, Netherlands) and stored at -80 °C until use. For immunohistological experiments, cryosections (10 μm thick) were cut and mounted on Super Frost slides (Fischer Scientific, Pittsburgh, PA).

Cell cultures: A human corneal epithelium (HCE) cell line transformed with SV 40 (ATCC/CRL11135) was cultured under standard conditions (5% CO_2, 95% humidified air, 37 °C) in Dulbecco's modified Eagle's medium with Ham's nutrient mixture F-12 (DMEM-F12) supplemented with 5% fetal calf serum, 5 μg/mL of insulin, 0.1 μg/mL of cholera toxin, 50 mg/mL of streptomycin, 50 IU/mL of penicillin, 0.5 mg/mL of epithelium growth factor, and 0.5% DMSO.

Primary cell cultures of human corneal epithelium were established from donor corneas, obtained from the local eye bank. Briefly, limbus was dissected from five human corneas, and cut into small pieces. Explants were transferred to 25 mL culture flasks, left for 2 h to enable adherence of epithelial cells to the support, and finally cultured as previously described for HCE.

Human corneal keratocytes cell cultures were established from five donor corneas. Briefly, a 8 mm diameter corneal button was punched out and the Descemet's membrane with attached endothelium was stripped off. The epithelium was also totally removed by alcohol treatment and scraping. The naked residual stroma was then cut into small pieces and treated with 0.2% collagenase B (Roche Diagnostics, Meylan, France) for 3 h at 37 °C. After centrifugation, the pellet of keratocytes was resuspended in DMEM-F12, supplemented with 10% FCS, and 1% each of glutamine, penicillin, streptomycin, and amphotericin B, and further subcultured. During subculture, keratocytes derived fibroblasts were detached using a 0.1% trypsin/EDTA solution.

A human corneal endothelial cell line (HCEN) was kindly provided by Prof. K. Engelmann and Dr. J. Bednarz, Hamburg, Germany. This cell line was obtained by transfection with the coding gene for the large T protein (LT) of the oncogenic DNA simian virus 40 (SV40) [13]. HCEN reproduced the morphological, and functional characteristics of normal endothelium, and was cultured under standard conditions (5% CO_2, 95% humidified air, 37 °C) in 50% M199 and 50% Ham's F12 supplemented with 10% fetal calf serum, 2% glutamine, 50 mg/mL of streptomycin, 50 IU/mL of penicillin, 20 μg/mL ascorbic acid, and 20 μg/mL of insulin. A human epithelial conjunctival cell line (Wong-Kilbourne derivative of Chang conjunctiva, clone 1-5c-4, ATCC CCL-20.2) was cultured under standard conditions (5% CO_2-95% humidified air, 37 °C) in Eagle's minimal essential medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 mg/mL streptomycin, and 50 IU/mL penicillin. The online ATCC catalog states that CCL-20.2 cells were originally thought to be de-

Table 1. Sequence of primers used for human KLFs and target genes amplification

Gene	Sequence (5'-3')	Product size (bp)	GenBank number
KLF1	F: AGAGGATCCAGGTGTGATAG R: CAGTCACTAGGAGAGTCCAA	369	NM_006563
KLF2	F: CCAAACTGTGACTGGTATTT R: CCCACCTGTCTCTCTATGTA	349	AF134053
KLF3	F: AGAACACACACAGGAGAAAA R: CTGACCATATGGAAGTTTTG	352	XM_003452
KLF4	F: GTTTTGAGGAAGTGCTGAG R: CAGTCACAGTGGTAAGGTTT	332	XM_047516
KLF5	F: AGACCAGTTCTTCACTGACA R: ATTAAGAGTGTCCATTGCTG	360	NM_009769
KLF6	F: ACCCGGCCCGACATGGACGTG R: CAGGCTGTTGTTCTCTAAGTT	323	XM_046278
KLF7	F: CCTCCACATGAAGAGACATA R: CGGGAAATAATTCCAATAGT	356	NM_003709
KLF8	F: CTTTTGGCTAGTGATTTCAG R: AACAGTAGGAATGTTTGCTG	194	NM_007250
KLF9	F: GAAGTCACTGCTCTTTGGTC R: AAGCATAAAGGGCCTAGAAT	349	XM_005584
KLF10	F: CAGAAGTCAGTGTTGGTCTC R: TGACTCCTTATCCTTGATGA	315	NM_005655
KLF11	F: CAGTGTTCATCACCTCTAGC R: AAGCAGCAAACTTTTTATCA	180	NM_003597
KLF12	F: CAGTATCTTCAGCGTCATCT R: GTCACATTTAGCAGGTCATC	352	NM_007249
KLF13	F: ATCCTAGCGGACCTCAAC R: CCTGTGTGAGTTCTCAGGTG	361	NM_015995
KLF15	F: GTTGGGTATCTGGGTGATAG R: TCTCCTCCAGAAACTCTTCA	397	NM_014079
ENDOGLIN	F: GCCGTGCTGGGCATCACC R: CAGTCTCTCCTGCTGGGC	373	NM_000118
ODC	F: ACTGCCACTTCCTCGATG R: AAGGGTCTTCACGATGGC	212	NM_004152

For each Krüppel-like factor (1 to 15) and the target genes (endoglin, ornithine decarboxylase/ODC), the nucleotide sequences of the two specific primers are shown. The "Product size" is the size of the amplification product. The gene accession used to design the primer pairs is listed.

rived from normal conjunctiva, but subsequent analyses suggest that they are HeLa cells. HeLa cells have some desirable properties as a generic epitheloid cell line that are advantageous to the present study. Each cornea led to independent primary cell cultures. Purity and identity of all the established cell lines and primary cultures were confirmed using established markers in immunological or RT-PCR assays. Molecular and immunological assays were always performed at the 5th subculture of all the cultures. Homogeneous state of confluence (80%) was also respected.

Cell transfection: The mammalian expression vector containing the cDNA of the human KLF4 was subcloned into pMT3 (Invitrogen, San Diego, CA) and kindly provided by Dr. Anil K. Rutsgi, University of Pennsylvania, Philadelphia, PA. The construct was verified by the ability to produce KLF4 protein with the appropriate molecular size using western blot experiments on cytoplasmic extracts of COS-7 cell line. The cells were trypsinized 16 h before transfection. Cells were grown to 80% confluence in 100 mm culture dishes. Transient transfections of 2 μg of empty pMT3 or pMT3-KLF4 and 2 μg of pCMV-β-galactosidase were performed by a liposome mediated method using Lipofectamine™ 2000 according to manufacturer's instructions (Invitrogen, Cergy-Pontoise, France). For each transfection sample, 4 μg of DNA and 60 μl of Lipofectamin™ 2000 were, respectively diluted in 1.5 mL of DMEM-F12 medium without serum. After 5 min incubation, the diluted DNA and Lipofectamine™ 2000 were combined and the mix was incubated for 20 min at room temperature to allow the DNA-Lipofectamin™ 2000 complexes to form. Three mL of complexes were added to each well. After 6 h of transfection, the medium with complexes was removed and the classic medium (supplemented with 5% fetal calf serum, 5 μg/mL of insulin, 0.1 μg/mL of cholera toxin, 50 mg/mL of streptomycin, 50 IU/mL of penicillin, 0.5 mg/mL of epithelium growth factor, and 0.5% DMSO) was added. After 36 h transient transfection, total mRNA of one part of the culture, was extracted and submitted to RT-PCR experiments using oligonucletide primers specific for KLF4, endoglin, and ODC. The other part was used to determine the β-galactosidase activity (normalization of transfection) using a high sensitivity β-galactosidase assay kit (Stratagene Europe, Amsterdam, Netherlands). Our previous work showed that the transfection percentage of HCE cell line was 57% with this protocol.

RT-PCR experiments: Total mRNA was extracted from human total cornea, conjunctiva, and cell cultures using Trizol (Invitrogen, Cergy-Pontoise, France). The cDNA was generated using Superscript First-Strand Synthesis System for RT-PCR (Gibco-BRL, Cergy-Pontoise, France). Specific oligonucleotide primers were originally generated by using the web program Primer3 based on the published full length human mRNA sequences of each specific gene; and designed to avoid genomic DNA amplification (Table 1). All the primers were preliminary checked to be able to amplify their corresponding KLFs, using human tissue already described to express these KLFs (positive control). PCR amplification was carried out in an Eppendorf Mastercycler (Eppendorf France; Le Pecq, France), using 50 ng of total mRNA per reaction and according to the following program: initial denaturation at 95 °C for 5 min, followed by denaturation at 95 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 30 s (35 cycles), terminated by a final extension of 72 °C for 7 min. The PCR products were separated on a 2% agarose gel and sequenced on both strands to confirm the specificity of the reaction, using the same primers, the DNA Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Courtaboeuf, France) and the Applied Biosystems model 377 DNA Sequencer. Amplification of the housekeeping gene GAPDH was used as positive control. A negative control for amplimer contamination was done using a complete PCR reaction mix. Band intensities were analysed by densitometry using NIH image (version 1.63) and normalized against that of the GAPDH band.

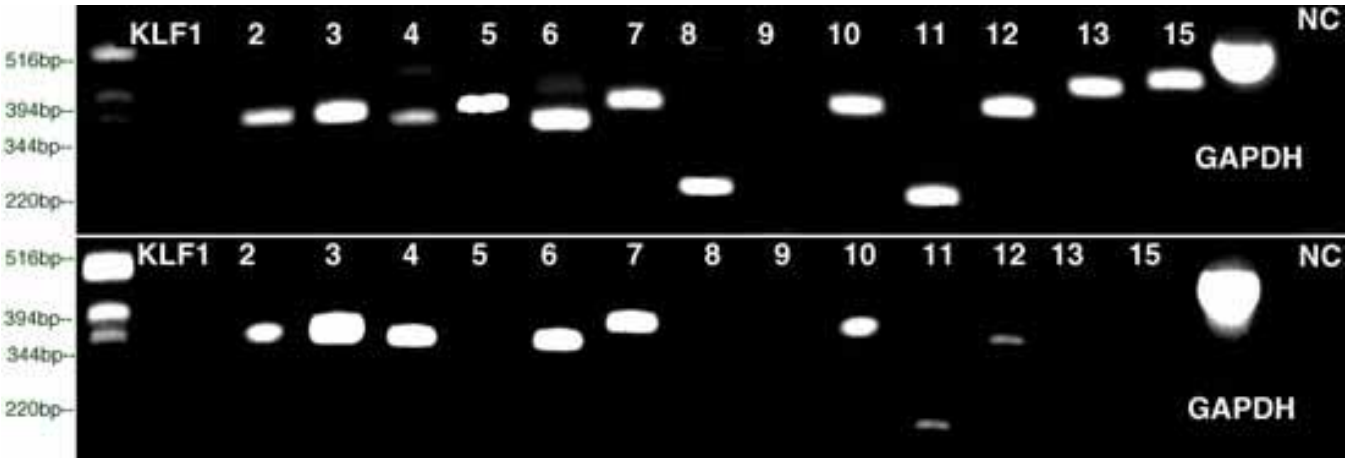

Figure 1. KLF expression pattern in human cornea and conjunctiva. The expression pattern of the different KLFs was determined by RT-PCR on total mRNA extracted from 5 human total cornea (upper panel) and 10 conjunctiva (lower panel) using specific primers to generate KLF1 to 15 products. Positive control (GADPH) was performed under the same conditions but using oligonucletide primers specific to the housekeeping gene GADPH (434 base pairs). Negative control (NC) was performed in the absence of oligonucleotides or matrix.

Immunohistological and cytological experiments: Cryosections of whole cornea and cells grown in Lab-Tek culture chambers (MC2, Clermont-Ferrand, France), were fixed in 4% paraformaldehyde in PBS (pH 7.4) at 4 °C for 1 h, rinsed three times with PBS, and incubated in PBS with 5% bovine serum albumin (Sigma Aldrich, St Quentin Fallavier, France) at 25 °C for 30 min. Cells and tissues were incubated overnight at 4 °C in the presence of an anti-KLF4 polyclonal (1/300 dilution in PBS; Santacruz product number 1905; Tebu, Le Perray en Yvelines, France). This was followed by three washes with PBS; a 1 h incubation in the presence of a goat FITC conjugated anti-rabbit antibody at room temperature and again three washes with PBS. Finally, after DAPI (diluted 1/500 in PBS) nuclear staining for 1 min, samples were examined under a Zeiss Axiophot microscope(Zeiss France; Le Pecq, France).

RESULTS

KLF transcripts are expressed in human cornea and conjunctiva: To determine the expression pattern of the human KLFs in native cornea and conjunctiva, classical RT-PCR experiments were performed using total mRNA isolated from the five human corneas, the ten human conjunctivas and the human KLF specific primers (Figure 1, Table 2). Reproducible results were obtained in both tissues. Under our conditions, we never detected KLF1 (Erythroid KLF) and KLF9 (Basic Transcription Element Basic 1) transcripts. In contrast, transcripts encoding twelve out of fourteen KLFs were identified in the cornea, and eight of these were also identified in the conjunctiva. The eight KLFs expressed simultaneously in both tissues were KLF2 (Lung KLF), KLF3 (Basic KLF), KLF4 (Gut KLF), KLF6 (Core Promoter Binding Protein, Zf9), KLF7

Table 2. Tissue and cell KLFs expression patterns in human ocular surface

	KLF													
	1	2	3	4	5	6	7	8	9	10	11	12	13	15
Total cornea	-	+	+	+	+	+	+	+	-	+	+	+	+	+
Human corneal epithelial cells (Primary culture)	-	+	+	+	+	+	+	+	-	+	+	+	-	+
Human corneal epithelial cells (Established cell line)	-	+	+	+	+	+	+	+	-	+	+	+	-	+
Human corneal keratocytes (Primary culture)	-	+	+	+	-	+	+	+	-	+	+	+	-	+
Human corneal endothelial cells (Established cell line)	-	+	+	+	-	+	+	+	-	+	+	+	+	+
Total conjunctiva	-	+	+	+	-	+	+	-	-	+	+	+	-	-
Human conjunctival epithelial cells (Established cell line)	-	+	+	+	-	+	+	-	-	+	+	+	-	-

RT-PCR experiments were conducted on total mRNA extracted from whole cornea, whole conjunctiva, and cells constituting these two ocular surface tissues. The data presented here summarize results obtained from the use of 5 corneas and 10 conjunctival tissues. For all the cells, the presented results are obtained from 5 different mRNA extractions and RT-PCR amplifications. Negative and positive amplifications are symbolized by "-" and "+", respectively.

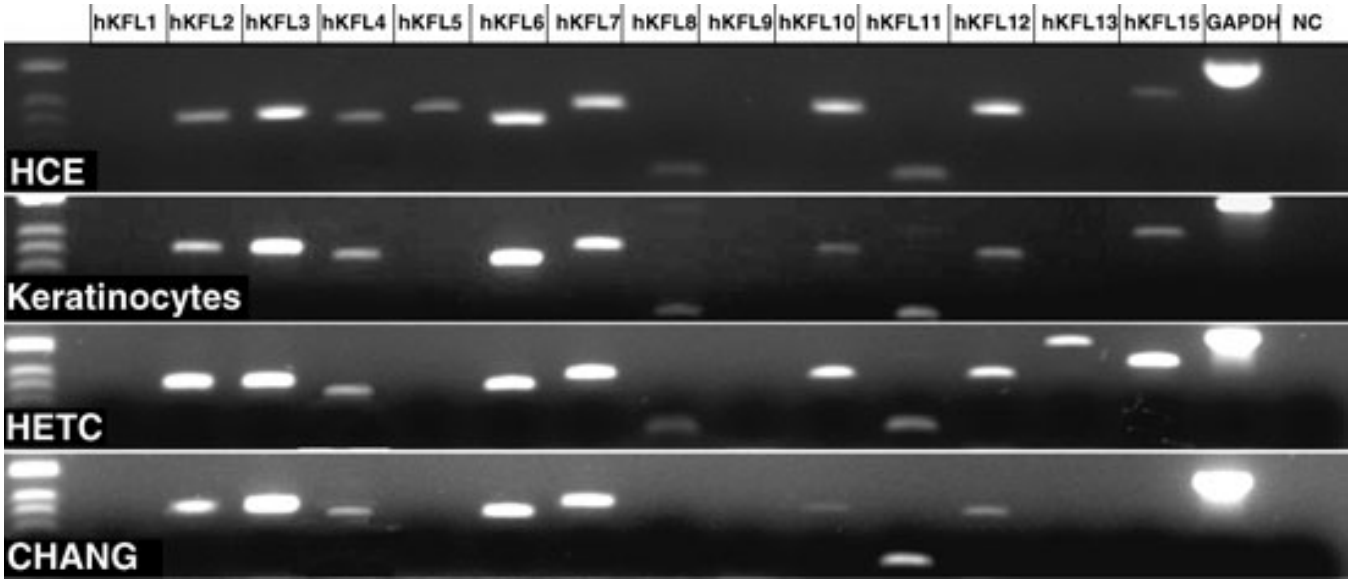

Figure 2. Cellular expression patterns of human KLFs in cornea and conjunctiva. The expression pattern of the different KLFs was determined by RT-PCR on total mRNA extracted from human corneal epithelial cell line (HCE); keratinocytes; human cornealendothelial cell line (HTEC); human epithelial conjunctival cell line (CHANG). Positive control (GADPH) was performed under the same conditions but using oligonucletide primers specific to the housekeeping gene GADPH. Negative control was performed in the absence of oligonucleotides or matrix.

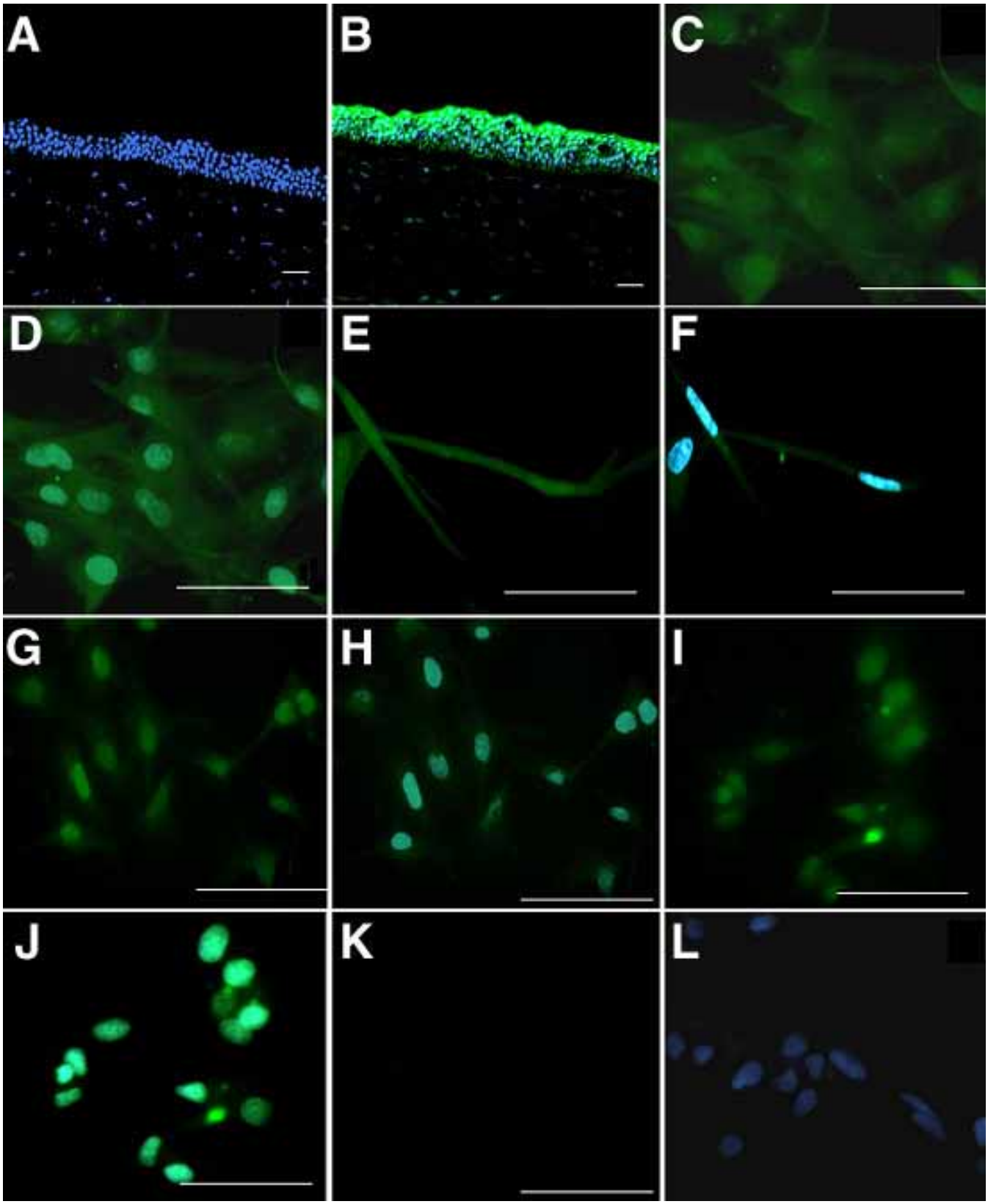

Figure 3. Cell and tissue immunolocalization of KLF4 proteins in human ocular surface. KLF4 immunolocalization (green fluorescence staining) was performed in human cornea (**B**), HCE cell line (**C**,**D**), human corneal keratocytes (**E**,**F**), HTEC cell line (**G**,**H**), and human conjunctival epithelial cell line (**I**,**J**). Negative controls (**A**,**K**) were obtained with the same conditions but in the absence of KLF4 antibody incubations. Cell nuclei were visualized with DAPI (blue staining; **A**,**B**,**D**,**F**,**H**,**J**,**L**). Acquisitions were made under standard conditions with a fluorescence Axiophot microscope (Zeiss). Magnifications for **A** and **B** are x40, and for **C**-**L** are x200. Scale bar represents 10 μm.

(Ubiquitous KLF), KLF10 (TGF β early gene 1), KLF11 (TGF β early gene 2) and KLF12 (AP-2 repressor; Figure 1).

These initial data led us to better determine the KLF expression patterns in corneal and conjunctival compartments at the cellular level. The major cells constituting the cornea and conjunctiva were analyzed using primary cultures or established cell lines (Figure 2, Table 2). Ten KLFs (KLF2, KLF3, KLF4, KLF6, KLF7, KLF8, KLF10, KLF11, KLF12, and KLF15) were expressed commonly in corneal epithelial cells, in endothelial cells, and in keratocytes. KLF5 was only detected in epithelial cells and KLF13 was only detected in corneal endothelial cells. Both epithelial primary cell culture and established cell line (HCE) expressed the same KLFs suggesting that the HCE cell line could be used as a model to study the KLFs' biological roles in the corneal epithelium. The eight KLFs previously detected in total conjunctiva were all found in human conjunctival epithelial cells.

KLF4 proteins are present in human ocular surface: We found KLF4 to be strongly expressed in the epithelial part of the human cornea, but both stroma and endothelium were also marked (Figure 3B and Figure 4). KLF4 immunolabeling also appeared in primary cultures of corneal keratocytes (Figure 3E,F), corneal epithelium (data not shown); and in corneal epithelial (Figure 3C,D), endothelial (Figure 3G,H), and conjunctival epithelial cell lines (Figure 3I,J). In all the different cell cultures, we found positive nuclear and cytoplasmic staining (Figure 3C-J), suggesting a translocation of KLF4 between protein synthesis (cytoplasm) and molecular action (nucleus) sites.

KLF4 over-expression downregulates endoglin and ornithine decarboxylase (ODC) expression in human corneal epithelial cells: KLF4 transient over-expression has been shown to reduce ODC and endoglin mRNA levels, in different cellular models [14,15]. RT-PCR experiments using HCE total

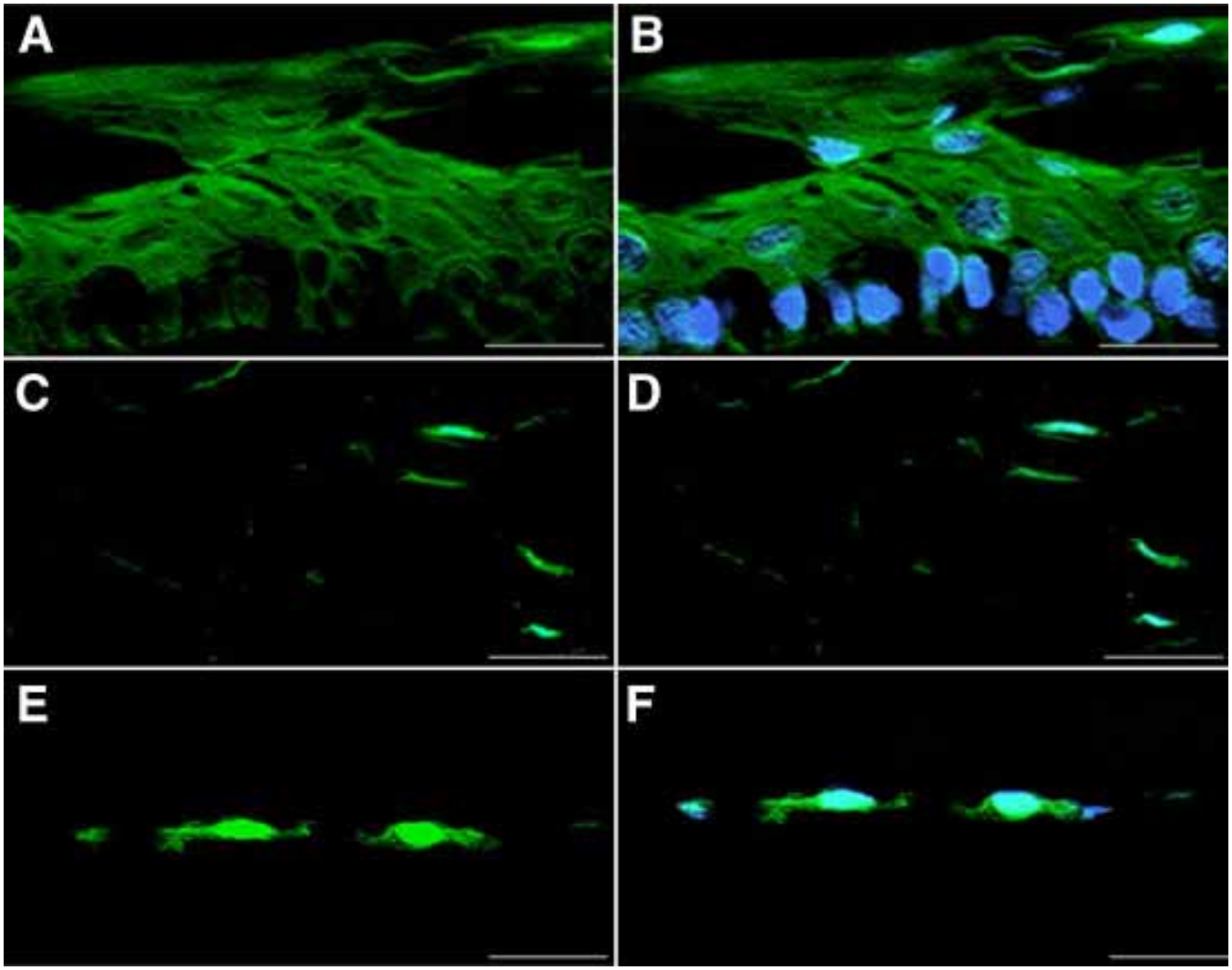

Figure 4. Cell and tissue immunolocalization of KLF4 proteins in human ocular cornea. KLF4 immunolocalization (green fluorescence staining) was performed in human corneal epithelium (**A**,**B**), corneal stroma (**C**,**D**), and corneal endothelium (**E**,**F**). Cell nuclei were visualized with DAPI (blue staining). **A**,**C**,**E**: Green only images related to KLF4 proteins. **B**,**D**,**F**: Green-blue images related to mix of KLF4 and DAPI staining. Acquisitions were made under standard conditions with a fluorescence Axiophot microscope (Zeiss). Magnification is x400. Scale bar represents 10 μm.

mRNA indicated the co-expression of KLF4 together with ODC and endoglin (Figure 5A). In order to verify KLF4 biological activity in HCE cells we performed KLF4 transient transfection experiments. Due to the KLF4 endogenous expression, its transient transfection resulted only in a 2.12 fold increase in KLF4 messenger accumulation (Figure 5B). Nevertheless, this over-expression was systematically correlated (five independent experiments) to a 2.27 and 1.77 fold reduction, respectively, for the ODC and endoglin mRNA levels.

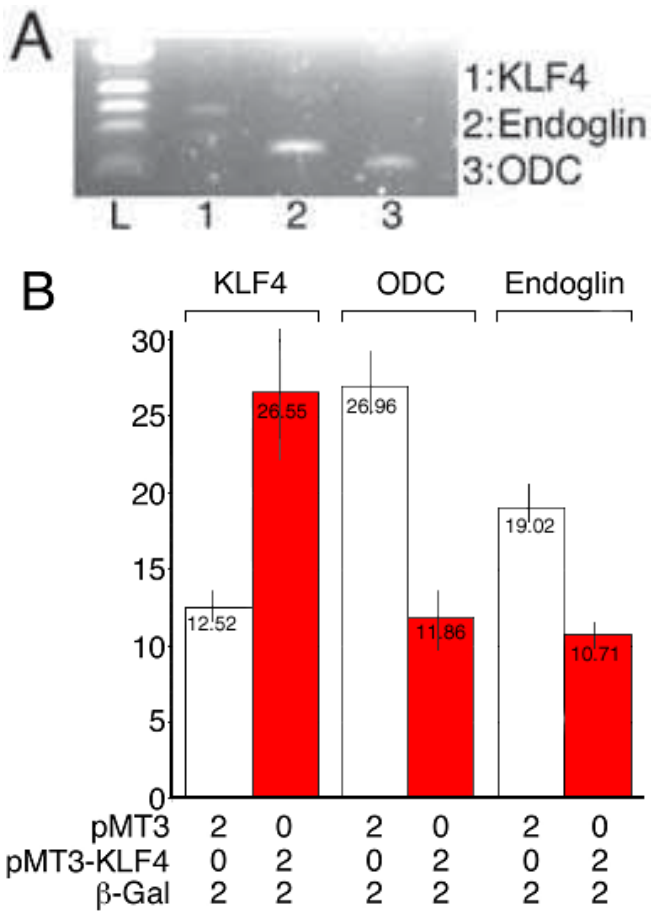

Figure 5. KLF4 transient transfection in HCE represses endoglin and ODC gene expression. **A**: Detection of the messengers encoding KLF4, endoglin and ODC by RT-PCR on total mRNA extracted from human total cornea. **B**: HCE cells were transfected with 2 μg of pMT3 or 2 μg of pMT3-KLF4 vector and 2 μg of pCMV-β-galactosidase. After 36 h transient transfection, total mRNA was extracted and submitted to RT-PCR experiments using oligonucletide primers specific of KLF4, endoglin, ODC and GAPDH. Fold induction or reduction of KLF4, endoglin and ODC mRNA levels were normalized using GAPDH mRNA levels. Determination of the β-galactosidase activity was realized to rationalize with the transfection percentage efficiency. The data presented were obtained from five independent cultures and PCR amplifications. They are shown with their mean ± standard deviations of five independent assays. KLF4 was normalized with the efficiency percentage based on the simultaneous determination of β-galactosidase. ODC and endoglin levels and variations are similarly reported normalized to β-galactosidase enzymatic activity.

DISCUSSION

The aim of the present study was to determine the expression pattern of the different KLF transcription factors in the human conjunctiva and cornea in order to better define their respective implications in human ocular surface. The KLFs are evolutionary determinants of vertebrate development controlling the formation of several organ systems, cell proliferation, and differentiation [2-4]. We demonstrated that a majority of KLFs are present in the corneal and/or conjunctival areas where they may participate in mechanisms fundamental to the conservation of the integrity of the ocular surface. Strong redundancy was observed between the patterns of the 14 KLFs established in cornea and conjunctiva, simultaneously expressing 12 and 8 KLFs, respectively. Note, that the co-expression of such a high number of KLFs was already reported in the placenta [16]. This redundancy of KLF expression in eye could be one of the explanations of the absence of ocular phenotype in all of the postnatal KLF mutant mice currently generated and analyzed. In addition, a strong similarity was also observed in terms of KLFs ocular expression patterns between human and mouse cornea, arguing for an important role of KLFs in the eye development and physiology. In fact, six KLFs (KLF3, 4, 5, 6, 13, 15) were also detected in postnatal mouse cornea by SAGE experiments [12]. The conservation of KLFs expression between mouse and human may argue for an important role of these six KLFs in the eye development and physiology.

KLF4 was originally cloned from a NIH3T3 library and a primary mouse embryonic fibroblast library [8]. It was described to be expressed in regions of the gastrointestinal tract, testis, skin, thymus, and lung [17-19]. We detected the expression KLF4 transcripts and proteins in every compartment of the ocular surface. This new localization in the human ocular surface completes recent data indicating that KLF4 is expressed after the 10th embryonic day in the mouse developing eye and post natal cornea (Personal communication, H. Sakai, May 2004) [12,20]. Interestingly, the ocular surface is the second tissue together with the gastrointestinal tract, where KLF4 persists during adult life after expression in embryonic life [21].

Its expression in both conjunctival and corneal epithelium is in agreement with previous data describing KLF4 as an epithelial zinc finger highly enriched in the population of terminally differentiated, post-mitotic epithelial cells [22]. KLF4 expression in the conjunctival endothelial cells completes its published localization in vascular endothelial cells [23]. Cytoplasmic and nuclear staining was observed for KLF4 at cellular levels of the human ocular surface, highlighting the KLF4 translocation between these two cellular compartments. The ability of KLF4 to circulate between nucleoplasm and cytoplasm has already been reported [24]. Indeed, nuclear localization signals have been reported for KLF4 [25].

We showed that transient transfection of KLF4 in HCE cells significantly inhibited ornithine decarboxylase and endoglin gene expression. Ornithine decarboxylase involved in cell proliferation and differentiation was similarly shown to be downregulated in HT-29 colon cancer cells by KLF4

overexpression [14,26]. Endoglin is a component of the transforming growth factor β receptor system involved in vascular remodeling that was identified to be downregulated in the RKO colon cancer cell line [16,27-29]. These results suggest a conservation of KLF4 biological activities in these epithelial cell lines and present the HCE cell line as being a good model for further studies regarding KLF biological implications. In addition, we found (data not shown) the co-expression of KLF13 with its target gene RANTES, in corneal endothelial cells, which are strongly related to immunological functions of cornea. These data are very interesting because RANTES has been described to modulate T lymphocyte activation [30]. Similar molecular regulation could occur in the cornea using the KLF13/RANTES pathway.

KLF4 is required for terminal differentiation of goblet cells in the colon [10] and it mediates p53 dependent G1/S cell cycle arrest [22,31]. Co-localization of p53 and KLF4 in the ocular surface raises the possibility that KLF4 might have an important function in regulating corneal and conjunctival growth and proliferation [8,23]. Similarly to its role in the intestine, KLF4 may act as a corneal growth arrest associated, epithelial specific gene (inhibition of DNA synthesis and involved in terminal differentiation of specific epithelial tissues) or as a differentiation promoting gene product responsible for activating downstream genes required for the differentiated epithelial phenotype [14].

In conclusion, we established for the first time, the cell and tissue specific expression patterns of the Krüppel-like transcription factors in the human ocular surface. We showed that one of these factors, KLF4, conserved its capacity to repress the expression of endoglin and ODC. Our results are the first answer to the questions concerning the implications of KLF4 in cornea, raised by Norman et al. [12], after their discovery of high expression of KLF4 in the normal postnatal mouse cornea [12]. Due to its physiological capacities in terms of cellular control and target genes, further studies are necessary to define precisely the true role of KLF4 during embryonic and adult life in the ocular anterior segment. Abnormal expression (decrease or absence) of KLF4 contributes to hyperproliferation and malignant transformation [32], raising the question of pathophysiological models implicating KLF4 in human eye disease.

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