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Olfactomedin-like 3 (OLFML3) gene expression in baboon and human ocular tissues: cornea, lens, uvea and retina

Iràm Pablo Rodríguez-Sánchez^{1,2}, Maria Lourdes Garza-Rodríguez¹, Karim Mohamed-Noriega³, Venkata Saroja Voruganti⁴, Maria Elizabeth Tejero⁵, Ivan Delgado-Enciso⁶, Diana Cristina Perez Ibañez¹, Natalia E. Schlabritz-Loutsevitch⁷, Jibrán Mohamed-Noriega³, Margarita L. Martínez-Fierro⁸, Diana Reséndez-Pérez⁹, Shelley A. Cole⁴, Humberto Cavazos-Adame³, Anthony G. Comuzzie⁴, Jesús Mohamed-Hamsho³, and Hugo Alberto Barrera-Saldaña^{1,2,§}

¹Universidad Autónoma de Nuevo León, Departamento de Bioquímica y Medicina Molecular, Facultad de Medicina, Monterrey, Nuevo León, México

²Vitaxentrum. Blvd. Puerta del Sol 1005, Colinas de San Jerónimo, Monterrey, Nuevo León, México

³Universidad Autónoma de Nuevo León, Departamento de Oftalmología, Hospital Universitario “Dr. Jose Eleuterio Gonzalez”, Monterrey, Nuevo León 64460, México

⁴Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX, USA

⁵Instituto Nacional de Medicina Genómica, Periférico sur 4124. Torre Zafiro II Col. Ex Rancho de Anzaldo, México, DF, México

⁶Facultad de Medicina, Universidad de Colima, Avenida Universidad 333, Colonia las víboras, CP 28045; Instituto Estatal de Cancerología de la Secretaría de Salud del Estado de Colima. Colima, Col

⁷The University of Tennessee, Health Science Center, Obstetrics and Gynecology, E102 Rout Center for Women and Newborns

⁸Universidad Autónoma de Zacatecas, Laboratorio de Medicina Molecular, Unidad Académica de Medicina Humana y Ciencias de la Salud, Carretera Zacatecas-Guadalajara Km,6, Ejido la Escondida

⁹Universidad Autónoma de Nuevo León, Departamento de Biología Celular y Genética, Facultad de Ciencias Biológicas, Monterrey, Nuevo León, México

Abstract

Background—Olfactomedin-like is a polyfunctional polymeric glycoprotein. This family has at least four members. One member of this family is OLFML3, which is preferentially expressed in placenta but is also detected in other adult tissues including the liver and heart. However, the orthologous rat gene is expressed in the iris, sclera, trabecular meshwork, retina, and optic nerve.

Methods—*OLFML3* amplification was performed by RT-PCR from human and baboon ocular tissues. The products were cloned and sequenced.

§Corresponding author: Dr. Hugo A. Barrera-Saldaña, Universidad Autónoma de Nuevo León, Facultad de Medicina, Departamento de Bioquímica y Medicina Molecular. Av. Madero y Dr. Aguirre Pequeño, Col. Mitras Centro, Monterrey, Nuevo León, México 64460. Tel.: +52 81 83294173 x2591; fax: +52 81 83337747. habarrera@gmail.com.

Results—We report OFML3 expression in human and baboon eye. The full CDS has 1221 bp, from which a OFR of 406 amino acid was obtained. The baboon *OLFML3* gene nucleotidic sequence has 98%, and amino acidic 99% similarity with humans.

Conclusions—*OLFML3* expression in human and baboon ocular tissues and its high similarity make the baboon a powerful model to deduce the physiological and/or metabolic function of this protein in the eye.

Keywords

Olfactomedin; eye; Old World Monkey; gene expression; animal model; corneal vascularization

INTRODUCTION

The Olfactomedin-family (OLF) is a group of extracellular glycoproteins that contain a domain in its C-terminal region [22]. This domain has about 250 amino acids and is named the OLF domain [6, 22]. The members of this family (seven diverse groups [26]) share different physiological functions between them. They seem to play a regulatory role in vertebrate embryogenesis [3] and other specific functions [26]. OLF includes noelins, implicated in neuronal differentiation during development, neurogenesis, and generation of the neural crest [3, 13–14]; tiarin, a dorsalizing factor of the neural tube [24]; amassin, a mediator of rapid intercellular adhesion [7]; myocilin, which has a role in trabecular meshwork inducible glucocorticoid response [10, 20] [mutations in the OLF domain of myocilin have been associated with primary open angle glaucoma (POAG) [1, 12]]; optimedin, which is expressed in the retina and the trabecular meshwork of the human eye and that has been suggested to be involved in disorders of the anterior segment of the eye and the retina[23]; and latrophilin, which is associated with cell adhesion and signal transduction[21].

Olfactomedin-like (OLFML) glycoproteins are other members of OLF and consist of at least five different molecules. OLFML1 regulates cell proliferation by increasing the percentage of cells in the S phase. OLFML2A and OLFML2B are known as photomedins. The former is expressed selectively in the outer segment of photoreceptor cells and the latter in all neurons of mice retina[6]. The *OLFML3* gene, which has been suggested to have a physiological role as a matrix-related component involved in placental and embryonic development in human and mouse and that may be involved in eye disorders [22, 27].

In humans the *OLFML3* gene encodes a 1852 nucleotide messenger RNA (mRNA), and contains a coding DNA sequence (CDS) of 1221 nucleotides flanked by two untranslated Regions (UTRs) (UTR-5' with 119 nucleotides and UTR-3' with 522 nucleotides). The gene has three exons (exon-1: 223 nucleotides, exon-2: 286 nucleotides and exon-3: 1352 nucleotides) separated by two introns and spans 2.9-kilo base pairs (kbp) in chromosome 1 at band p13.1. The Open Reading Frame (ORF) predicts a protein of 406 amino acids and 44 kilo Daltons (kDa)[27]. Expression of this gene has been reported in multiple human tissues, such as placenta, liver, heart, skeletal muscle, small intestine, lung, kidney, colon, thymus, spleen, and the brain [27], but not in the eye. On the other hand, OLFML3 has been identified by shotgun proteomics in rabbit corneal endothelium proteins [11].

The precise role of OLFML3 remains elusive. However, the orthologous rat gene of the human *OLFML3* (HNOEL-iso) gene is expressed in the iris, sclera, trabecular meshwork, retina, and the optic nerve [2, 22].

We report for the first time, the expression of OFML genes in human eye tissues [endothelial layer of the cornea, uvea, lens, retina and retinal pigment epithelium (RPE)].

We also found expression of the *OFML3* gene in cornea, lens, iris and RPE tissues of the baboon [*Papio hamadryas* (*PHA*)], an Old World Monkey (OWM).

MATERIALS AND METHODS

Animal model

Animal procedures were performed according to ethical guidelines and were reviewed by the Institutional Animal Care and Use Committee of the Texas Institute of Biomedical Research (TIBR). Animals were maintained at the Southwest National Primate Research Center in San Antonio, Texas at TIBR. All the animals shared the same diet and environmental conditions before sample collection. All baboons are gang-housed and fed *ad libitum* on a standard low-fat chow diet (Harlan Teklad 15% Monkey Diet, 8715). Ocular pieces (n= 3) from three male baboons were collected at scheduled necropsy. Eyepieces were frozen in liquid nitrogen until dissection and nucleic acid extraction. The age of the baboons was 6, 9, and 11 years. Each ocular piece was dissected and the cornea, iris, lens, sclera, anterior chamber, posterior chamber, vitreous, retina-RPE and optic nerve were separated.

Human biological specimens

The study protocol was approved by the Health Research Ethics Board of the Medical School of the Universidad Autonoma de Nuevo Leon (UANL). Biopsies from five human eyes were collected at the Department of Ophthalmology of the Hospital Universitario “Dr. Jose Eleuterio Gonzalez” of the UANL. All patients voluntarily agreed to participate in the study and signed an informed consent. The specimens came from programmed enucleations or any other eye surgery procedure where ocular tissues were removed as part of the patient’s treatment. Biological samples were collected immediately after tissue removal and were immersed in RNAlater solution (Ambion Inc., Austin, TX). The samples were transported at 4°C to the laboratory and stored at –70°C until use. All tissues were from male patients. Characteristics of the tissues are shown in Table 1.

RNA isolation from ocular tissues

Total RNA was extracted from the eye tissues samples with Trizol[®] reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). RNA was treated with RQ1 DNase (Promega, Madison, WI) for 15 min at 37°C to remove traces of genomic DNA. For the assessment of RNA purity and integrity, we used standard methods of spectrophotometry and gel electrophoresis, respectively.

Reverse transcription and Polymerase Chain Reaction

Retrotranscription (RT) reactions were carried out with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) and oligo (dT)12–18 Primer (Invitrogen) using 0.25 mg of total RNA from each tissue in 25 µL final volume. We used the online tool Primer3 (v. 0.4.0) to design primers that amplify *OLFML3* related transcripts [8]. Primers were designed based on UTRs of *OLFML3* mRNA sequences of *Macaca mulatta* (MMU) and *Homo sapiens* (HSA). Amplification reactions were carried out using 5 µL of cDNA (from RT reaction), 10 µM of each primer [sense hybridizing 13 bp before ATG initiation triplet (5′-CCTTCTACTCTGGCACCCT-3′) and antisense hybridizing 133 bp after TAG termination triplet (5′-GGAGCTGCAAGAATTTGATT-3′)] and the commercial kit PCR master mix from Qiagen (Valencia, CA). The amplification reaction design was for a final volume of 25 µL and it was carried out in a thermal cycler (Veriti 96-Well Thermal Cycler, Applied Biosystems). The amplification program used was as follows: an initial denaturation step of 4 min at 94°C, 40 cycles of 30 sec each at 94°C, 30 sec at

58°C, 90 seg at 72°C and finally an elongation step of 6 min at 72°C. The amplification products were visualized on 1% agarose gels stained with ethidium bromide and visualized under UV light (see Figure 1).

Molecular cloning, sequencing, and sequence analysis

The amplified products were cloned in the 3.5 kb-XL-TOPO vector and transformed into electrocompetent *Escherichia coli* bacteria strain Top 10 according to the manufacturer's specifications (Invitrogen). Positive clones were sequenced using Big Dye terminator cycle sequencing kit v3.1 using specific primers and/or M13 universal primers. The reactions were analyzed in the ABI PRISM 3100 Genetic Analyzer using the Sequencing Analysis Software v5.3 (Applied Biosystems). The sequences obtained from clones were aligned with human orthologous gene [GenBank: NM_020190] using the CLUSTAL W program followed by manual corrections in case of need. Both, the nucleotidic and amino acid sequences were deposited in the GenBank database (*PHA*-OLFML3: KC237060 and *HSA*-OLFML3 KC237062) [16].

RESULTS

RT-PCR, molecular cloning and sequence analysis

Amplification of the *OLFML3* nucleic acid species was performed by PCR using cDNA from different ocular tissues and *OLFML3* specific primers (see Figure 1). The amplicon size was as expected (1405 bp) and no other bands were seen as possible isoforms. In *HSA* only the RT-PCR for corneal endothelium, uvea, lens and retina-RPE was positive. In baboon tissues, the RT-PCR for cornea, lens, iris and retina-RPE was positive. The *PHA* and *HSA* mRNA *OLFML3* sequences were deposited in the GenBank database. The obtained amplified products were cloned and the sequences revealed the typical *OLFML3* mRNA organization and a full CDS of 1221 bp, from which an ORF of 406 amino acids was predicted (see Figure 2-A). The *PHA* *OLFML3* CDS nucleotidic sequence has 98% similarity with the *HSA* orthologous. The 23 nucleotidic changes for the 2% difference include: 19 synonymous and 4 non-synonymous changes. In the amino acidic sequence, the similarity between baboon and humans was 99% with only four amino acid changes: in amino acid position 10, there was a Leu in *PHA* and in *HSA* there was a Phe; in amino acid position 18, there was a Pro in *PHA* and a His in *HSA*, in amino acid position 232, there was a Gly in *PHA* and a Asp in *HSA*, and in amino acid position 235, there was a Met in *PHA* and a Leu in *HSA*.

The same *OLFML3* transcript was found expressed in the four analyzed tissues of both the *PHA* and *HSA* samples. The nucleotidic and amino acidic similarity was 98% and 99%, respectively. Based on these similarities and concordances we suggest that the baboon could be a good model to further explore and analyze the function of *OLFML3* in human eyes [4, 18]. We identified *OLFML3* expression in four human ocular tissues, which may provide a better understanding of this protein family.

DISCUSSION

Zeng et al., in 2004 reported *OLFML3* gene expression in several human tissues but not in human eye [27]. Other analyses in rabbit corneas identified a peptide that shows high similarity to the peptide coded by the *OLFML3* gene. In previous reports (human, baboon and rabbit), the metabolic function of *OLFML3* remains unknown. A new finding in humans suggests that the *OLFML3* gene may play a role in fetal development because this gene was found in the embryonic side of the placenta and not in the endometrial side [27]. However, there is no evidence suggesting its possible role in the eye thus far.

To our knowledge this is the first report of the expression of the *OLFML3* gene in human ocular tissues (corneal endothelium, lens, uvea and retina-RPE). In ocular tissues (cornea, lens, iris and retina-RPE) of *PHA* primate, we found an *OLFML3* transcript type, corresponding to the human isoform 1 (~1852 bp). It has been reported in humans that the ORF encoded by the *OLFML3* gene contains a 21 amino acid α -signal peptide followed by a coiled-coil domain of 77 amino acids at its N-terminal, topped with an OLFML domain at the C-terminal of 268 amino acids. The ORFs predicted for the new baboon OFLML3 using the nucleotidic sequence coincide in all its components (see Figure 2-A). In the polypeptide sequence there are two potential sites susceptible to N-glycosylation and disulfide bonding, the latter being conserved in the *PHA* ORF but differing in two positions at the signal peptide and two positions in the OLFML domain. Sites susceptible to N-glycosylation and disulfide bridges predicted by Ling-Chun Zeng and colleagues are conserved [27]. The OLF genes have a very diverse physiological role [22] and many of these genes are expression in the human eye [15]. Some mutations in these genes (e.g., myocilin and olfactomedin-2) are related with eye diseases, for instance, POAG [5, 9]. In mouse models (transgenic betaB1-crystallin-MYOC mice) overexpression of myocilin in the aqueous humor resulted in modification of the expression of multiple genes, including the *OLFML3* gene, which showed a decrease of expression of 0.72 times. Changes in gene expression of these genes suggest that myocilin and other proteins from the OLF have a role in modulating cellular adhesion [17]. In this paper we show the *OLFML3* gene structure and the presence of mRNAs in ocular tissues of baboons and humans. To confirm the presence of the OLFML3 protein in these tissues it is important to perform additional experiments such as immunohistochemistry and Western blot.

It is well known that several pro and anti-angiogenic mechanisms work together modulating eye vascularity. Likewise, maintaining avascularity of the cornea, regulating normal vascularization in the retina and choroid and regulating vascular proliferation are essential in maintaining homeostasis of the eye [19]. In animal models it was recently reported that some members of the OLF might have angiogenic effects on eye tissues. On June 10, 2010 some siRNA against *OLFML3* transcripts and antibodies against OLFML3 and OLFML3 polypeptide were patented for use as mediators of angiogenesis. These siRNA and antibodies could be useful in the treatment of diseases with pathologic vascularity [25].

In this study, we demonstrate *OLFML3* expression in humans and baboon eye tissue. Given these findings, and taking into consideration that some olfactomedins have a physiological role in angiogenesis [15] and with the new development of siRNA against *OLFML3* transcripts to treat diseases with altered vascularity [25], we suggest that OLFML3 may play a possible angiogenic role in ocular tissues and might participate in anterior segment and retinal diseases.

It is important to point out that more work is needed to identify the biologic effect of OLFML3 on eye tissues. We could infer that blocking, modulating or stimulating the action of OLFML3 could be an innovative treatment for different eye diseases. But much more work is needed to clarify its actual role.

Finally, expression of the *OLFML3* gene in human and baboon ocular tissues and the high similarity between them make the baboon a powerful model to deduce the physiological and/or metabolic function of this protein in the endothelial layer of the cornea, lens, iris/uvea and retina-RPE. Moreover, it would be important in the future to dig deeper into the role of these genes in normal and pathological physiology with tools such as antisense RNA assays and transgenic models.

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LIST OF ABBREVIATIONS

OWM	Old world primates
PHA	<i>Papio hamadryas</i>
HSA	Homo sapiens
b	base
bp	base pairs
PCR	Polymerase Chain Reaction
RT	Reverse Transcription
mRNA	messenger RNA
CDS	coding DNA sequence
UTR	untranslated region

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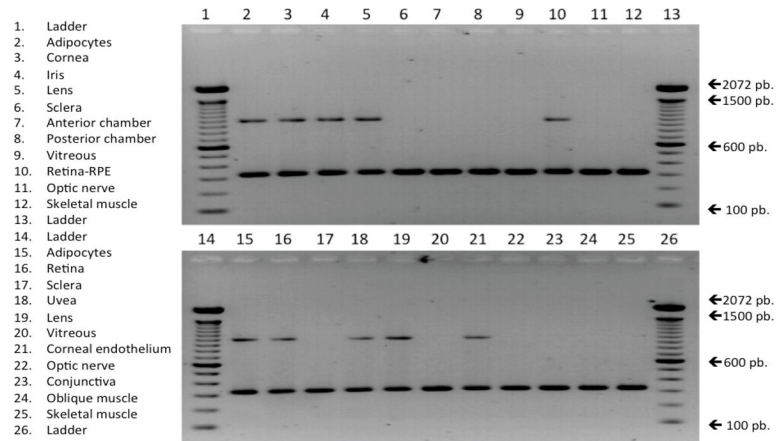
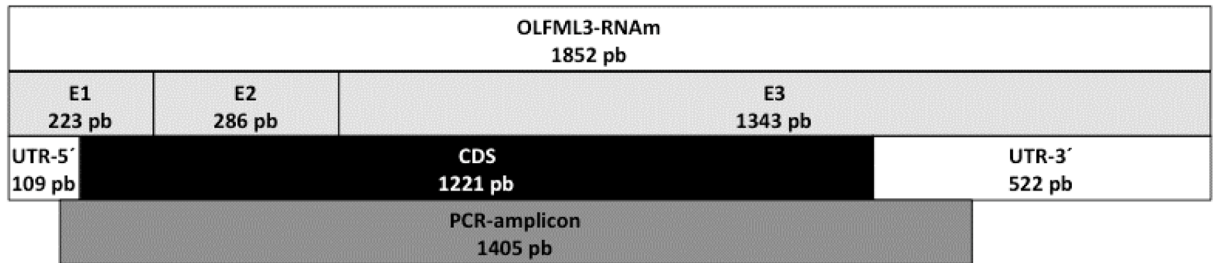


Figure 1. Electrophoresis analysis of the OLFML3 PCR amplicons

Lines 2 to 12 correspond to baboon cDNAs and lines 15 to 25 correspond to human cDNAs. The ladder consists of 15 blunt-ended fragments between 100 and 1500 bp in multiples of 100 bp and an additional fragment at 2072 bp (Invitrogen).

A



NOTES: E: exon; CDS: Coding DNA sequence

B

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PHA-OLFML3  mgpstpllifllswsghlqgQQHHLVEYMERRLAALAEERLAQCQDQSSRHAAELRDFKN 60
HSA-OLFML3  mgpstpllifllswsghlqgQQHHLVEYMERRLAALAEERLAQCQDQSSRHAAELRDFKN 60
*****

PHA-OLFML3  KMLPLLEVAEKEREALRTEADTISGRVDRLEREVDYLETQNPALPCVEFDEKVTGGPGTK 120
HSA-OLFML3  KMLPLLEVAEKEREALRTEADTISGRVDRLEREVDYLETQNPALPCVEFDEKVTGGPGTK 120
*****

PHA-OLFML3  GKGRNKEYDMVTDCGYTISQVRSMKILKRFGGPAGLWTKDPLGQTEKIYVLDGTQNDTA 180
HSA-OLFML3  GKGRNKEYDMVTDCGYTISQVRSMKILKRFGGPAGLWTKDPLGQTEKIYVLDGTQNDTA 180
*****

PHA-OLFML3  FVFPRLRDFTLAMAARKASRVRVFPFVWGTGQLVYGGFLYFARRPPGRPGGDGELENTLQ 240
HSA-OLFML3  FVFPRLRDFTLAMAARKASRVRVFPFVWGTGQLVYGGFLYFARRPPGRPGGGEMENTLQ 240
*****

PHA-OLFML3  LIKFHLANRTVVDSSVFPAEGLIPPYGLTADTYIDLAADEEGLWAVYATREDDRHLCIAK 300
HSA-OLFML3  LIKFHLANRTVVDSSVFPAEGLIPPYGLTADTYIDLAADEEGLWAVYATREDDRHLCIAK 300
*****

PHA-OLFML3  LDPQTLDTEQQWDTPCPRENAEAFVIGTLVYVVYNTRPASRARIQCSFDASGTLTPERA 360
HSA-OLFML3  LDPQTLDTEQQWDTPCPRENAEAFVIGTLVYVVYNTRPASRARIQCSFDASGTLTPERA 360
*****

PHA-OLFML3  ALPYFPRRYGAHASLRYNPRERQLYAWDDGYQIVYKLEMRRKKEEEV 406
HSA-OLFML3  ALPYFPRRYGAHASLRYNPRERQLYAWDDGYQIVYKLEMRRKKEEEV 406
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Figure 2. Sequence analysis of the *OLFML3* gene from HSA and PHA

A. – *OLFML3* gene anatomy. B. – Aligned aminoacidic sequences of PHA and HAS *OLFML3*. Lowercase sequence corresponds to signal peptide; italicized letter corresponds to coiled-coil domain and underlined letter to olfactomedin-like domain; highlighted in grey are potential sites susceptible to N-glycosylation and highlighted in black is the disulfide bond.

Table 1

Characteristics of human eye tissues.

Patient Age (yrs)	Diagnosis / Procedure	Eye Tissues collected
47	Enucleated eye for a traumatic penetrating injury	Retina, sclera and uvea
82	Penetrating keratoplasty and cataract extraction for a cornea stroma scar and dislocated cataract.	Lens, vitreous and corneal endothelium
71	Enucleated eye for an orbit carcinoma, infective keratitis and neovascular glaucoma	Optic nerve
7	Strabismus surgery (Medial Rectus Recession)	Conjunctiva
13	Strabismus surgery (Inferior oblique muscle myectomy)	Inferior oblique muscle, conjunctiva and tenon.