·Basic Research·

Expression of junctional adhesion molecule -1 in human corneal epithelium

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Abstract

• AIM: To investigate the expression and distribution of junction adhesion molecule-1 (JAM-1) in human corneal epithelium and compare with those of occludin.

• METHODS: The expression in RNAs of JAM-1 and occludin was revealed by RT-PCR and the presence of protein was analyzed by the FACS method. Double immunofluorescent staining was used to determine the tissue distribution of JAM-1 and occludin in human corneal epithelium.

• RESULTS: The expression of JAM-1 and occludin was found in cultured human corneal epithelial cells. The double immunofluorescent study showed positive staining for JAM-1 at cell borders in the entire epithelial layer, while relatively extensive staining was seen in the superficial layer, where it coexisted with the expression of occludin.

• CONCLUSION: JAM-1 is expressed in entire layer of human corneal epithelium encircling the cells.

• KEYWORDS: junction adhesion molecule-1; occludin; tight junction

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INTRODUCTION

The cornea which is found in the outermost part of the eye serves as the frontal barrier of the whole eyeball. It provides a biodefense barrier to the ever-changing external environment, protecting the eye not only from biological and chemical abuse, but also from penetration of tear fluid and its chemical constituents. Four types of intercellular junctions have been identified in the corneal epithelium: gap junctions, desmosomes, adherent junctions, and tight junctions (TJs). TJ functions as the seal between cells, forming the primary barrier to restrict the free diffusion of fluids, electrolytes, molecules and cells through paracellular pathways. TJ plays a central role in the regulation of paracellular permeability, and is also crucial in the generation and maintenance of cellular polarity in endothelial and epithelial cells. Under ultrathin section electron microscopes, TJs appear as a series of kissing points between the abutting cells, where the intercellular space is completely obliterated[1].

TJ components can be divided into four categories: peripherally associated scaffolding proteins, signaling proteins, proteins regulating membrane vesicle targeting, and transmembrane proteins [1]. Human junctional adhesion molecule-1 (JAM-1), also referred to as JAM-A, is the most recently identified transmembrane protein component. It was found to be among the first TJ-associated proteins appearing at cell-cell contacts during junction formation [2]. JAM-1 plays an important role in recruiting and localizing signaling complex to sites of cell-cell adhesion, promoting the formation of TJs [3]. It is also involved in leukocyte migration, platelet activation, angiogenesis and reovirus binding [4]. On the human cornea, TJ was detected in the superficial layer of corneal epithelium and corneal endothelium. Many reports revealed that several TJ-related proteins such as occludin, claudin and ZO-1 were detected in human corneal epithelial cells[5]. However, the expression of JAM-1 in human cornea remained unreported. The current study was designed to study the expression of JAM-1 and its distribution in human cultured corneal cells and corneal tissue.

MATERIALS AND METHODS

Antibodies Mouse anti-human monoclonal antibody to JAM-1 and rabbit polyclonal antibody to occludin obtained from Hycult biotechnology b.v. (Uden, Netherlands) and

from Zymed (South San Francisco, CA), respectively.

Reverse Transcriptase Polymerase Chain Reaction Human corneal epithelial cells (HCEC) purchased from Krabo were cultured to subconfluency with Epilife medium. Total RNA was isolated from cells using Trizol reagent (Invitrogen). Reverse transcription was carried out with the SuperScript™ First-Strand Synthesis System (Invitrogen). Approximately 0.3μg of total RNA was used in each reverse transcription reaction (the final volume was 20μL). Duplex PCR for JAM-1 and occludin was performed using 1μL of reverse transcription reaction in a 20μL reaction using *Taq* enzyme and buffer. The following primers were used for detection of JAM-1: 5'-GGTCAAGGTCAAGCTCAT-3' (forward), and 5'-CTGCATTTGCCTTACTCAG-3'(reverse), PCR products 581bp; occludin: 5'-TCAGGGAATATCCAC CTATCACTTCAG-3' (forward), and 5'-GTGAAGAGTAC ATGGCTGCTGCTGATC-3'(reverse), PCR products 136bp, respectively. The PCR reaction was carried out as follows: initial denaturation at 94℃ for 2 minutes, followed by denaturation at 94℃ for 60 seconds, annealing at 60℃ for 60 seconds, and polymerization at 72℃ for 60 seconds. The reaction was carried out over 35 cycles and completed by an extended polymerization at 72℃ for 10 minutes. After completion of the PCR amplification, 10μL of each PCR products were examined by 15g/L agarose gel and stained with ethidium bromide (0.5g/L).

Flow Cytometric Analysis After harvest in PBS, cultured human corneal epithelial cells were incubated with the primary antibody for 30 minutes on ice and washed twice with cold PBS, then incubated with 100mL/L human serum for 15 minutes, followed by washing with cold PBS twice. After incubation with secondary antibodies for 30 minutes on ice and washing, flow cytometry analysis was performed.

Immunofluorescence Colocalization of JAM-1 and occludin was studied by double immunochemistry. Cryostat sections (4μm in thickness) of the cornea were initially air-dried at room temperature for 1 hour and then fixed in chilled acetone for 10 minutes, followed by a subsequent blocking of nonspecific staining using serum-free Protein Block (Dako Cytomation California, USA) for 10 minutes. The sections were then incubated with mouse anti-JAM-1 antibody (1∶300) for 1 hour at room temperature in a moist chamber, washed, and then exposed to Alexa594 conjugated goat anti-mouse IgG (1∶500, 1 hour, room temperature). Then the sections were exposed to the rabbit polyclonal anti-occludin antibody (1∶300) at room temperature for 1 hour, and subsequently to Alexa488 conjugated goat anti-rabbit IgG (1∶500) for 1 hour at room temperature. After mounting, the sections were observed and photographed. Primary antibodies were omitted in negative controls.

RESULTS

Total RNA was extracted from cultured human epithelial cells and RT-PCR was used to determine the expression of JAM-1 and occludin transcript with a size of 581bp and 136bp. When the amplification reaction after 35 cycles, expression of JAM-1 and occludin was detected (Figure 1). Flow cytometry showed that JAM-1 was expressed on the surface of cultured human corneal epithelial cells (Figure 2). Immunofluorescence study showed that occludin immunoreactivity was largely limited to the uppermost superficial layer. The staining pattern between wing cells was punctate, no staining at the junction between column basal cells was

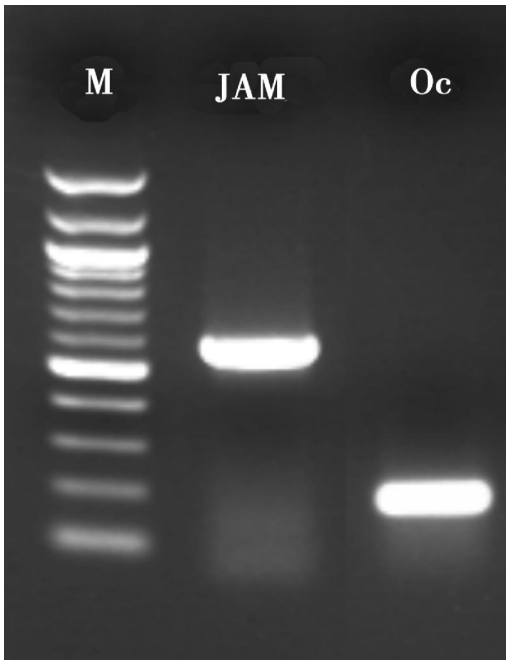

Figure 1 RT-PCR analysis of JAM-1 (JAM) and occludin (Oc) expression Expression in mRNAs of JAM-1 (581bp) and occludin (136bp) was detected. A 100bp ladder was used as m.w. marker (M)

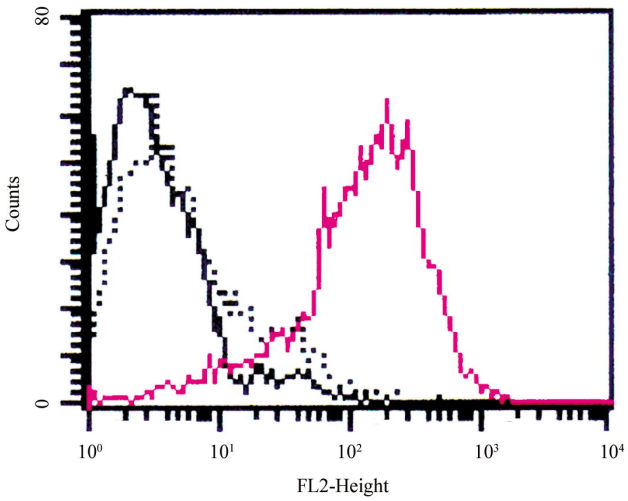

Figure 2 Human corneal epithelial cells were analyzed by flow cytometry for the surface expression of JAM-1 with anti-JAM-1 mAb Corneal epithelial cells staining with anti-JAM-1 antibody is shown in pink, and staining with negative control antibody in black

observed. While staining for JAM-1 was detected at cell borders in the entire epithelium, intense labeling was observed in the superficial layer, coexisting with that of occludin (Figure 3).

DISCUSSION

JAM-1, 36-41kDa, belong to a subgroup of the Ig super family [6]. It is composed of two extracellular Ig-like domains, a membrane-distal V-Ig-domain and a membrane-proximal C2-Ig-domain, followed by a single transmembrane segment and a short cytoplasmic tail. Its cytoplasmic domain interacts with several TJ-associated proteins such as occludin, ZO-1, AF-6, MUPP1, PAR-3 and cingulin [7-10]. JAM-1 appears at cell-cell contacts during junction formation [2]. Occludin was the first reported integral membrane protein of TJ[11], and was considered as a reliable and sensitive marker of the barrier function of TJ. The results of our study indicate that the JAM-1 is expressed by human corneal cells, and the expression is consistent with that of occludin in the superficial layer of the cornea epithelia, suggesting that it plays a role in the corneal epithelial barrier.

In this study, we found that JAM-1 expressed in the human corneal epithelial cells. The presence of this protein was confirmed by FACS study. Its mRNA was detected by RT-PCR. Double fluorescent staining study showed that the immunoreactivity of occludin was restricted in the superficial layer of the cornea. The positive staining for JAM-1 was at cell borders in the entire epithelial layer (superficial cells, wing cells and basal cells), while relatively intense immunoreactivity was seen in the superficial layer, wherein it coexisted with that of occludin. This result shows that JAM-1 is not only one of the components of TJ proteins, but also a membrane adhesion protein between corneal epithelial cells.

Although JAM-1 was first named as "junctional adhesion molecule", it is not TJ restricted. It was shown to be expressed in circulating cells such as neutrophils, monocytes, lymphocytes, red blood cells and platelets [12]. It is assumed that it plays an important role in host inflammatory response by influencing the leucocytes migration. Ostermann [13] showed JAM-1 as a ligand for integrin LFA-1. The binding of LFA-1 to JAM-1 after inflammatory stimulation can support neutrophil and memory T cell adhesion to JAM-1 and facilitate the leukocyte transmigration and diapedesis. The mAbs to JAM-1 can block monocyte infiltration both *in vivo* and *in vitro*. Our results showed that JAM-1 was expressed in the entire layer of epithelium, in places devoid of TJ, suggesting the speculation that besides TJ construction and regulation, it may also be involved in other functions in the cornea such as infection and graft rejection post-keratoplasty.

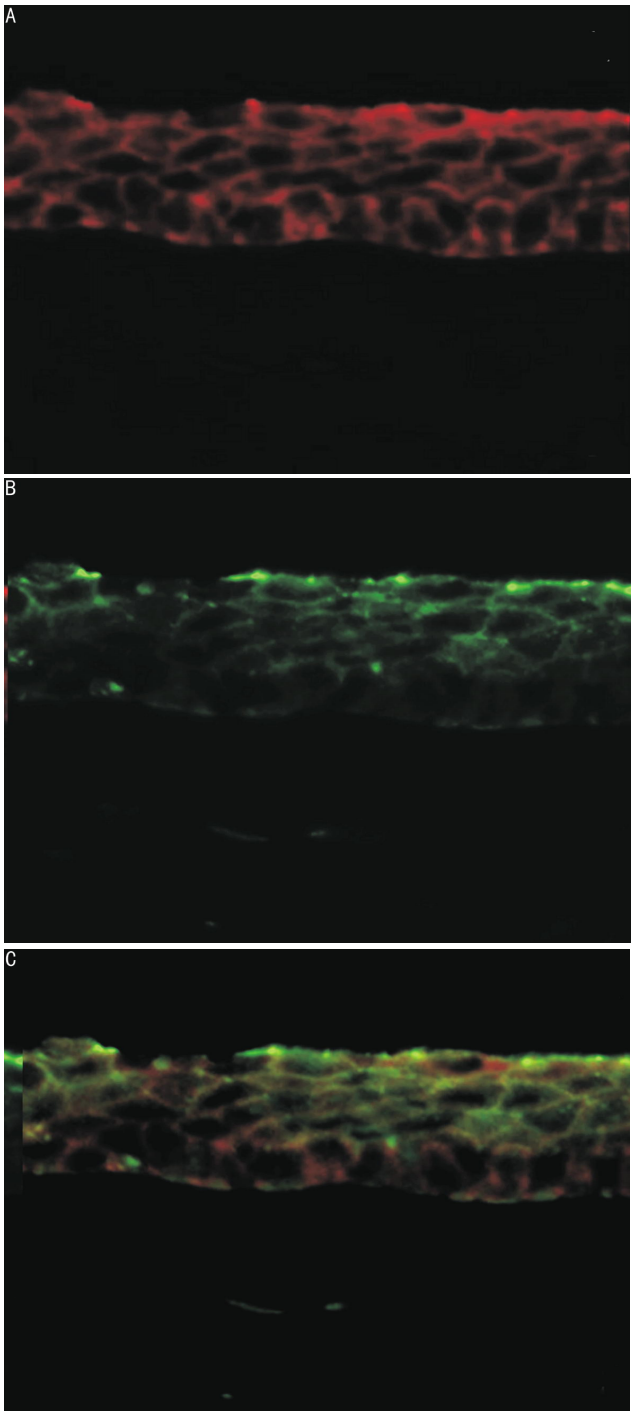

Figure 3 Immunoreactivity of JAM-1 (red) was present along the cell borders in the entire epithelial layer (A, C), whereas occludin (green) was mainly located in the superficial epithelial cells and the staining pattern between wing cells was punctate (B, C). The coexistence (yellow) of JAM-1 and occludin was observed in the merged image (C)

In summary, JAM-1 is the most recently identified transmembrane protein component that colocalizes with TJ components, and is believed to play an important role in the molecular architecture of TJ and recruitment of TJ components. It is also involved in leukocyte migration, platelet activation, angiogenesis and reovirus binding. In the

human cornea it coexists with occludin in the superficial epithelial layer, and also expresses at the cell border between basal column cells where TJ does not exist. This study revealed the presence of JAM-1 in the human cornea, but its function in physiologic and pathologic processes of cornea diseases needs to be further investigated.

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