Tight Junction Complex Profiles in Patients with Preterm Premature Rupture of the Membrane

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Abstract

Objective: We investigated the expression profiles of tight junction and adhesion molecules on the fetal membranes of patients with preterm premature rupture of the membrane (pPROM) compared with those of normal membranes.

Methods: We collected pPROM samples under institutional review board approval of Dankook University. Fetal membranes were collected and evaluated with immunocytochemistry for a qualitative pattern and by Western blot for quantitative expression of tight junction and adhesion molecules.

Results: We found significant changes in the properties of occludin as a tight junction for pPROM. The immunohistochemistry results showed that occludin lost polarization on the fetal membranes of patients with pPROM. The Western blot data showed that occludin expression decreased compared with that of normal membranes. Integrin beta1 expression decreased significantly in pPROM samples compared with that in normal samples.

Conclusion: The results suggest that occludin and integrin beta 1 signals are a major factor in human pPROM. More study is needed to determine how adhesion signaling is related to pPROM.

Keywords: Preterm premature rupture of the amniotic membrane; Inflammation; Fetal membrane; Occludin; Integrin beta1

Introduction

Rupture of the fetal membrane is detrimental during delivery and is caused by uterine contractions. However, rupture of the membrane precedes 30-40% of preterm premature births<37 weeks. A 1-2% risk of fatal death is included in this percentage and caused by factors such as neonatal complications. The risks of preterm premature rupture of the amnion (pPROM) are related to fetal distress, a prolapsed cord, abruptuon of the placenta, and infection. The main reason for pPROM is inflammation of the amniotic membrane due to infection of amniotic fluid and the reproductive organs [1,2]. pPROM is a major high-risk factor for premature delivery. The inflammation is related to a disturbance in the tight and adhesion junctions. However, the relationship between pPROM and the profiles of tight and adhesion junctions is unclear.

The amniotic membrane has three major sections called the amnion, chorion, and decidua [3]. The amniotic epithelium is connected to the epithelium through cell-cell tight junctions (TJ) and is bound to the basement membrane by several adhesion junction molecules, such as fibronectin, laminin, and glycosaminoglycans [4]. The amniotic epithelium has connections through apical TJ as a physical barrier to maintain the amniotic membrane [4]. The amniotic epithelial layer forms adhesion junctions with the chorion layer. TJs are an essential component of the differentiated epithelial cells required for polarized transport, intercellular integrity, and signaling for the connected amniotic barrier. A wide range of cellular systems can be used to learn how TJs are constructed and maintained and how they function. Adhesion junctions and molecules between epithelial and endothelial cells mediate the physiological roles of the amniotic membrane. Adhesion and TJ comprises two modes of cell–cell adhesion that provide different functions. Both TJ and adhesion junction complexes are associated with the actin cytoskeleton to maintain the amniotic membrane depending on the conditions. The apical junctional complex at the amniotic membrane is composed of TJ and adhesion junctions to stabilize the amniotic membrane barrier.

Inflammation of the amniochorion, called chorioamnionitis, is a major cause of pPROM [5]. Inflammation of the pPROM increases cytokine levels in the amniotic fluid because bacteria recruit cytokines, such as interleukins, from immune cells. These inflammatory cytokines activate several extracellular matrix (ECM) collagen-specific matrix metalloproteinases, resulting in matrix protein degradation of collagen [6]. Inflammation is linked with disruption of the epithelial cell–cell and epithelial cell–ECM interactions such as TJs on the fetal amniotic membrane. The relationship between inflammation of the pPROM and TJs has been clearly identified. However, the relationship between pPROM and adhesion junctions remains obscure. Adhesion junctions are related with adhesion molecules, such as focal adhesion kinase (FAK) [7]. Additionally, the integrin receptor is associated with adhesion junctions for adhesion to the ECM [8].

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Adhesion proteins and adhesion receptors, such as integrins, are involved in cell-to-cell adhesion and adhesion signaling [9]. There is no doubt that pPROM is related to cell-to-cell and cell to ECM interfaces [10]. Although pPROM is associated with TJs, the relationship between TJs and adhesion junctions is unclear. In particular, no study has investigated the relationship between TJs and adhesion junctions in the human pPROM. Therefore, we investigated the expression profile of adhesion junctions and molecules on the pPROM. TJs cause loss of adhesion signaling and induce apoptosis of fetal membrane epithelial cells.

We investigated the expression ratio of TJ proteins and the correlation between TJs and adhesion complex molecules, such as adhesion proteins and adhesion receptors (integrin), in the human pPROM.

Materials and Methods

Fetal membrane sampling from patients with pPROM

Normal and pPROM were obtained with informed consent of the mother. This experiment was approved by the Institutional Review Board of Dankook University Hospital, Cheonan, South Korea. The fetal membranes were collected within 1 hr postpartum. The membranes were washed with PBS for histological study (immunohistochemistry) and biochemical analyses (Western blot).

pPROM diagnosis

We diagnosed pPROM though a history of amniotic fluid leakage, amniotic fluid passing through the cervix or fluid accumulation in the posterior fornix, and by the nitrazine test paper reaction. Additionally, chorioamnionitis includes symptoms of maternal fever (>38°C), leukocytosis, fetal tachycardia, uterine tenderness, and foul-smelling amniotic fluid with no other source of infection.

The pPROM of patient 1 had a long latency period. We treated her with dexamethasone (6 mg intramuscularly four times at 12 h intervals) [11], antibiotic treatment, and body temperature, pulse, blood pressure, and uterine tenderness was checked daily. We also checked the white blood cell count, performed a non-stress test, estimated fetal weight, and assessed the amniotic fluid index (AFI) every week.

TJ immunohistochemistry

An immunohistochemical examination was conducted to localize and describe the TJ pattern on the epithelial cells of the fetal membrane. The fetal membranes were fixed in 4% paraformaldehyde for histological study. The fixed amniotic membranes were permeabilized with 1% Triton X-100 in PBS (pH 7.4) for 10 min and washed several times. After antigen retrieval, the samples were incubated overnight at 4°C. The primary antibodies were rabbit anti-zonula occludens-1 (ZO-1), occludin, and claudin-4 (1:200, Millipore, Milford, MA, USA) and describe the TJ pattern on the epithelial cells of the fetal membrane epithelial cells.

We incubated with 5 μM DAPI (Sigma, St. Louis, MO, USA) for 3 min at room temperature to visualize nuclei before mounting in Vector Shell (Vector Laboratories, Burlingame, CA, USA). The TJ pattern of the stained fetal membranes was imaged by confocal microscopy (M700 Zeiss, Oberkochen, Germany).

Western blots to quantify TJs, adhesion molecules, and integrin beta1 fetal membrane proteins

TJ proteins in the fetal membrane were quantitatively analyzed by Western blot with a TJ marker antibody. Two mg of fetal membrane homogenate was mixed with 2 ml protein extraction buffer (PRO-EXTRACT; Intron Biotech, Daejeon, Korea) in ice for 10 min. After completely dissolving the proteins, the extraction solution was centrifuged at 15000 rpm for 35 min. A 200 μl aliquot of the protein extract solution was boiled with 40 μl 5 × sample buffer (60 mM Tris-Cl pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, and 0.01% bromophenol blue) for 5 min. Twenty-five μl of boiled sample was loaded into each well of a gradient gel (Bio Rad, Hercules, CA, USA) and transblotted onto a nitrocellulose membrane for about 2.5 h at 350 mA. The membranes were blocked with 5% fat-free milk in Tris buffer solution with 0.5% Tween-20 (TBST) for 1 h at room temperature. The primary antibody was diluted 1:1,000 in 2% fat-free milk TBST solution and incubated with the membrane overnight in a cool chamber. We evaluated the membranes with the A-10 rabbit anti-FAK antibody, (1:1000, Santa Cruz Biotecnoiogy, Santa Cruz, CA, USA) and integrin beta1 antibody (1:500, mouse anti-integrin beta1 antibody, Santa Cruz Biotecnoiogy) as primary antibodies. The blotted membranes were washed with TBST and incubated with horseradish peroxidase-conjugated anti-mouse or rabbit secondary IgG. The immunoreactive bands were detected after 1 h using an enhanced chemiluminescent detection reagent (Western Bright ECL, K-12045; Advansta Corp., Menlo Park, CA, USA). Band intensities were analyzed using ImageQuant LAS4000 mini (GE Healthcare, Parsippany, NJ, USA). Experiments were performed in triplicate.

Table 1: Characteristics and profiles of the patients with preterm premature rupture of the membrane (pPROM)
Results

Clinical diagnostic profile of pPROM

Table 1 shows the normal and pPROM profiles. The normal group had no pPROM symptoms, whereas the two pPROM showed very low AFI values compared with those in the normal group. In particular, the pPROM of patient 2 was confirmed to be infected by a urea culture assay. The pPROM of patient 1 did not show evidence of an amniotic fluid infection.

Localization and distribution of TJ proteins on the pPROM

We performed immunohistochemistry to determine the localization and distribution of TJ proteins, such as ZO-1, occludin, and claudin-4, on the epithelial layer of the fetal membrane. Figure 1 shows the polarity and distribution of ZO-1 and claudin-4 on the membrane, which was not significantly different between normal and the pPROM-1 and 2 patient samples. However, the localization and distribution of occludin showed a slightly different pattern between the normal and pPROM samples. Occludin in the pPROM-1 and 2 samples showed less polarity and staining in the apical area compared with those of normal fetal membranes.

Profile of TJ proteins on pPROM

Figure 2a shows the Western blot results for the TJ protein expression levels of ZO-1, claudin-4, and occluding. The band intensities from Western blot and the protein expression ratios are shown in Figure 2b. As shown in Figure 2a and 2b, ZO-1 expression decreased significantly in the normal fetal membrane sample compared with that in the pPROM group. In contrast, the quantity of claudin-4 in normal samples was similar to in patient-2 but lower than that of patient-1. Occludin expression decreased significantly in the pPROM group (patients 1 and 2) compared with that in normal membranes.

Figure 2: Expression of tight junction proteins in fetal membranes obtained from patients (Normal: N, Patient-1: P-, and Patient-2: P-2) with preterm premature rupture of the membrane (pPROM) after cesarean section (CS). (a) The first line is a blot with the anti-zonula occludens-1 (ZO-1) antibody. The second line is fetal membrane homogenates prepared for Western blotting. A total of 17 µg protein was loaded in 25 µl in each lane. Results are representative of one experiment repeated three times. (b) Tight junction protein expression was quantified by densitometry and is expressed as mean peak optical density ± standard error of the number of samples in each group. The experiment was repeated three times, and band intensity is presented as mean ± standard deviation. Asterisks (*) represent a significant p-value between pPROM and control groups.

Focal adhesion protein and integrin beta 1 expression profiles on pPROM

We performed a quantitative analysis of integrin beta1, as an adhesion molecule receptor, and FAK, as an adhesion signal molecule, on the fetal membrane of patients with pPROM. FAK protein expression had a similar expression ratio in the normal and pPROM samples.
Discussion

We analyzed the qualitative and quantitative patterns of TJ and adhesion junction proteins on the pPROM. Patients with pPROM had significantly decreased occludin expression compared with that of normal patients. In contrast, claudin-4, as a TJ protein, increased significantly compared with that of other TJ proteins, such as ZO-1 and occludin (Figure 2a). This result suggests a relationship between TJs and adhesion junctions on the fetal membranes of patients with pPROM. Adhesion molecules (e.g., FAK) and adhesion signal receptors, such as integrin, decreased on the pPROM compared with that of normal membranes. In particular, fetal membranes of patients with severe pPROM showed decreased integrin receptor expression compared with that of normal patients. These results suggest that pPROM may involve TJs and adhesion junctions.

We investigated whether the TJ and adhesion junction protein profiles of pPROM were due to inflammation. TJs have no direct relationship with apoptosis of the amniotic membrane. We hypothesized that the integrin receptor, as an ECM receptor, mediates apoptosis in amniotic membrane cells, which brings about pPROM, as the pPROM membranes showed dramatically decreased integrin beta-1 expression. Integrin beta-1 is a common binding pair of the integrin dimer for microenvironmental responses of cells [8]. The integrin receptor recruits many secondary cytoplasmic molecules depending on microenvironmental signaling to mediate cell behaviors, such as proliferation, differentiation, survival, and apoptosis [9]. Interestingly, we found that the amniotic membrane in patients with pPROM had significantly decreased integrin beta-1 receptor expression.

An in vitro organ culture system in an animal pPROM study reported that the inflammatory condition decreases claudin-3 and claudin-4 levels at apical junctions [5]. However, our data show increased claudin-4 expression on the fetal membranes of patients with pPROM. Claudin is a transmembrane protein component among TJs, and 24 claudin subtype members have been reported in mice and humans [10]. In particular, claudin-4 is determined barrier properties, such as the blood barrier, for TJs. Claudin-4 is linked with intracellular molecules, such as ZO-1, and inflammation of the small intestine decreases claudin-4 expression [12]. Claudin-4 has one hairpin structure compared with that of occludin. Based on our results, occludin rather than claudin-4 is related with pPROM.

Occludin was first identified on TJ membrane proteins. Occludin has four transmembrane domains with a long carboxy terminal cytoplasmic domain and a short amino-terminal cytoplasmic domain. The physical function of occludin on the TJ strand has not been well explained. The role of occludin in epithelial cell TJs is related with other inflammatory diseases, such as liver infection caused by the hepatitis C virus [13]. Occludin mediates apoptosis by disturbing cell-to-cell TJs during inflammation [14]. Our results suggest that pPROM apoptosis may be related with occludin in the fetal membrane.

Epithelial cell-to-cell connections occur through a junctional complex that includes TJs and adhesion junctions. The TJs of apical epithelial cells form a junctional complex with adhesion junctions [9]. pPROM arises from what are likely multifaceted and multistep pathogenic pathways. The pathophysiological processes may involve both endogenous and exogenous fetal and maternal factors. However, adhesion junctions and signaling have not been included in the factors related to adhesion molecules and pPROM. Our data clearly show that integrin beta-1 is an adhesion signaling receptor related with pPROM. Adhesion molecules such as FAK are associated with cell-cell and cell-matrix adhesion signaling. FAK has several roles, such as proliferation, migration, and apoptosis [15]. However, FAK has not been associated with pPROM.

Taken together, the fetal membrane has TJs and adhesion junctions on the cell-to-cell interface. The role of the junctional complex as a TJ and adhesion junction at the region of inflammation of the fetal membrane requires more study.

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