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Knockdown of aquaporin 3 is involved in intestinal barrier integrity impairment

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ABSTRACT

AQP3 is a water/glycerol transporter expressed at the basolateral membrane of colonic epithelial cells. Although AQPs are expressed in the gastrointestinal tract, their effect on intestinal barrier has not been clear. Here, we showed that knockdown of AQP3 caused a dramatic, dose-dependent increase in *E. coli* C25 translocation, with the reduction of TEER and increasing LY permeability. Western blots revealed that expression of Claudin-1 and Occludin were significantly decreased in the AQP3 knockdown group, demonstrating that this treatment enhances paracellular permeability via an opening of the tight junction complex. These data not only describe the correlation between transcellular and paracellular pathways in human intestines, but also show that targeted knockdown of AQP3 might impair the intestinal barrier integrity.

1. Introduction

Intestine, an important digestive organ, plays an important role in maintaining the body's normal nutrition metabolism. It is generally accepted that the intestine may play an important role in trauma, surgery, burns, shock, etc. [1–2]. Many lines of evidence support the concept that trauma and shock may initiate a series of intestinal reactions such as intestinal cytokine response, translocation of intestinal bacteria, or even cause systemic inflammatory response syndrome (SIRS) and sepsis with subsequent multiple organ failure (MOF) [3–4]. The mechanism of intestinal mucosal barrier is very complicated, which may be involved in intestinal mechanical barrier, local biological factors, chemical factors and immune function. But the specific mechanism is still unclear [5].

Water is essential because it must be introduced to satisfy metabolic demands [6–7]. Studies have shown that large quantities of liquid and electrolyte transport and water channel proteins (aquaporins, AQPs) are closely related [8]. AQPs are a family of small (30 kDa/monomer) hydrophobic, integral membrane proteins, which are belonging to a special superfamily of membrane integral proteins called MIPs (major intrinsic proteins) [8–9]. The tight junction (TJ) is an intracellular junctional structure that mediates adhesion between epithelial cells and is important for epithelial cell function [10]. TJ constitutes the barrier both to the passage of ions and molecules through the paracellular pathway between an apical and basolateral cell membrane domains [10]. Claudins, Occludin, and junctional adhesion molecules are the major three transmembrane proteins at TJ [11–13]. AQP3 is mainly expressed in the epithelia of the upper and lower digestive tract [14]. Hara and Verkman found that skin barrier function was impaired in AQP3-deficient mice and glycerol transport by AQP3 was responsible for the functional defects in skin [15]. Thiagarajah proved the evidence for involvement of AQP3 in colitis and for impaired enterocyte proliferation in AQP3−/− mice [16].

The purpose of this work was to study the correlation between AQP3 and TJs and whether target knockdown of AQP3 could impair intestinal mucosal barrier integrity, thus leading to endotoxin translocation.

An in vitro model of AQP mediated bacterial translocation (BT) was developed to allow direct study of the interaction between enterocytes, AQP and translocating bacteria without the potential confounding factors. Our results showed that knockdown of AQP3 was associated with a reduction of Claudin-1 and Occludin expression and increasing of bacteria translocation.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), phosphate buffered saline (PBS), fetal bovine serum (FBS),non-essential amino acids solution (NEAA 10 mM, 100×), penicillin–streptomycin solution (10,000 units/ml penicillin and 10,000 μg/ml of streptomycin), trypsin–EDTA solution (0.05% trypsin, 0.53 mM EDTA) were obtained from Invitrogen-Life Technologies. Transwell polyester

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membranes (0.4 μm pore size) were purchased from the Costar Corning Corporation (NY, USA). Antibodies directed against AQP3 and tight junction proteins, anti-mouse and anti-rabbit IgG antibodies (FITC-labeled) were purchased from SANTA CRUSE (San Francisco, CA, USA).

2.2. Preparation of AQP3 knockdown Caco-2 cells

ShRNA of human AQP3 lentivirus gene transfer vector encoding green fluorescent protein (GFP) and puromycin sequence was constructed by Sunbio Medical Biotechnology Co., Ltd (Shanghai, China). The Lenti-scrambled-shRNA served as negative control. For shRNA of human AQP3, the oligonucleotide sequences were GGATATGATCAATGGCTTCTT. The aqp3shRNA was packaged with lentivirus following the manufacturer's protocols. When Caco-2 cells had grown to 90% confluence, the Lenti-scrambled-shRNA was infected along with separate infection of the LV-aqp3shRNA at a multiplicity of infection (MOI) of 50. There were three groups: control group (CON, normal Caco-2 cell), negative control group (NC), RNAi groups. Stable cell lines were selected with 5 μg/ml puromycin (Sigma–Aldrich) for one week. After that, cells were analyzed using Semi-quantitative PCR and Western blot for AQP3 expression.

2.3. Formation of the Caco-2 monolayer

Caco-2 cells from Chinese Academy of Sciences were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 1% non-essential amino acids, 1% L-glutamine and 1% penicillin–streptomycin mixture. Cells were kept at 37 °C in 5% CO_2 and 95% humidity. Once sufficient numbers of cells were grown, they were harvested by trypsinization (0.05% trypsin, 0.53 mM EDTA), washed, resuspended in DMEM. 2×10^5cells were seeded onto the apical chamber of Transwell bicameral chamber containing a 0.4 μm pore size member. The cells were grown for 14 days to allow them to reach confluence and fully differentiate. The medium was changed every other day.

2.4. Measurement of electrical resistance and paracellular permeability

The formation of a sealed monolayer of Caco-2 cells was monitored by measurements of the transepithelial electrical resistance (TEER) with a Millicell electrical resistance system (Millipore, Bedford, MA) [17]. The transepithelial electrical resistance (TEER) is an index of confluence and integrity of monolayers. Based on LY permeability as previous described [18], Monolayers were used in experiments only after the TEER had risen above 150 Ω/cm^2. Typically about 14 days of culture are needed to reach such a TEER. Measurements were performed at 37 °C and results were expressed as mean ± SD.

Transepithelial transport was studied with cells grown on permeable supports (Transwell) for 7, 14, 21, 28 days. The culture medium of apical and basolateral compartment was removed by aspiration, and monolayers were washed three times with HBSS at 37 °C, 30 min before the beginning of the experiment. Paracellular permeability was assessed by measuring the flux of LY (Sigma–Aldrich) from apical to basal chambers of the Transwell at the same time as BT. LY is a fluorescent dye and was used in these experiments as a marker of paracellular permeability [19]. LY was diluted in transport buffer (HBSS with Ca^{2+}, Mg^{2+}, +10 mM HEPES, pH 7.4) and added to the apical compartment at a final concentration of 50 μM. After three hours, LY concentration in the basolateral chamber was measured with an TECAN Infinite F500 (Männedorf, Switzerland) luminescence spectrometer against a standard concentration curve (excitation 427 nm, emission 536 nm).

2.5. Bacterial translocation

The DMED used to culture the cells was removed from the bicameral chamber and replaced with fresh HBSS. An inoculum of 10^2, 10^5 or 10^7 bacteria (*E. coli* C25) was added to the apical side of the Caco-2 cell monolayer that had been grown in culture for 14 days. BT was then measured by quantitatively culturing samples of medium (100 μl) obtained from the basal chamber at 20, 40, 120, and 180 min after the bacteria was placed in the apical chamber. As the aliquots were removed, new medium was added to the basal chamber. The number of viable translocation E.coli C25 was quantitated by pour late assay with Macconkey's agar. In all experiments the TEER was monitored at the beginning and end of the 3-h test period to assess the integrity of the Caco-2 cell monolayer.

2.6. Immunocytochemistry

For immunocytochemistry, slides were fixed in acetone and methanol (1:1) for 10 min and were then rinsed. After blocking endogenous peroxides and proteins, The fixed cells were incubated over night at 4 °C with phosphate-buffered saline containing anti-human AQP3 or the tight junction associated proteins primary Antibodies diluted 1:100 to 1:400 then incubated with respective horseradish peroxidase-labeled secondary Antibodies for 1 h at 37 °C and then color developed using diaminobenzidine solution for 10 min, and counterstained with hematoxylin.

2.7. Western blot analysis

For western blot, cells were reseeded in 6-well plates at a density of 0.2×10^6cells/ml with fresh complete culture medium. Cells with or without treatment were washed with cold PBS and lysed in ice-cold RIPA buffer (containing 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 0.25% sodium deoxycholate) with 1 mM NaF, 10 μM Na_3VO_4, 1 mM PMSF, and a protease inhibitor concoction (10 μg/ml leupeptin, 10 μg/ml aprotinin and 1 μM pepstatin). Cell lysates were incubated at 4 °C for 30 min. After centrifugation at 12,000 rpm for 10 min at 4 °C, protein concentrations were determined by bicinchoninic acid (BCA) protein assay. Then, 40 μg of proteins (for AQP3, Occludin, Claudin-1, Claudin-3, Zo-1 and GAPDH) were denatured in 5 × SDS–PAGE sample buffer for 5 min at 100 °C. The proteins were separated by 12.5% SDS–PAGE and transferred onto PVDF membrane (Millipore, Bedford, MA) for 30 min at 4 °C. Non-specific binding was blocked with 5% dry skimmed milk in TBST (20 mM Tris–HCl, 137 mM NaCl, 0.1% Tween 20, pH 7.4) for 2 h at room temperature. After blocking, membranes were incubated with specific antibodies against AQP3 (1:1000), Occludin, Claudin-1, Claudin-3 and Zo-1 (1:1000), and GAPDH (1:1000) in dilution buffer (2% BSA in TBS) overnight at 4 °C. The blots were incubated with HRP-conjugated anti-mouse or anti-rabbit IgG (1:2000) at room temperature for 2 h. Antibody binding was detected using an enhanced chemiluminescence (ECL) detection system following manufacturer's instructions and visualized by autoradiography with Hyperfilm.

2.8. Statistics

Results are expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to compare TEER, LY permeability, and the magnitude of BT after $\log_{10}$ transformation among RNAi, CON or NC group. A two-tailed paired *t* test was used to compare pre- versus post-experimental TEERs in the same monolayers. Statistical significance was accepted as $p < 0.05$.

3. Results

3.1. AQP3 expression in Caco-2 cell line and lentiviral transfection

First, we examined the expression of AQP3 at its mRNA and protein level by performing RT-PCR and western blotting analysis in Caco-2 cell lines. To further examine the role of AQP3 in the intestinal barrier function, RNAi experiments were performed. (Fig. 1) shows AQP3 mRNA and protein expressing after lentiviral transfection.

3.2. Effect of AQP3 on TEER and LY

Caco-2 cells were seeded on Transwell (0.4 μm pore size), the cells were grown for 14 days to confluence and full differentiation. As shown in Fig. 2, knockdown of AQP3 caused an increase in paracellular permeability in Caco-2 monolayers, as judged by a loss of TEER and increased LY permeability. These effects were time dependent, and both variables showed significant changes at 7, 14, 21 days in RNAi group compare with control group ($p < 0.05$) (Fig. 2A), whereas no significant changes between CON and NC group ($p > 0.05$). The results were also proved by LY permeability (Fig. 2B).

3.3. Effect of time and magnitude of E. coli on BT

As illustrated in Fig. 3, the magnitude of *E. coli* translocation across the Caco-2 monolayer was both dose and time dependent. Different inoculum (10^2, 10^5 or 10^7) were added to the Caco-2 epithelial monolayer system and the number of translocation bacteria measured at 20, 40, 120, and 180 min after inoculation. As the inoculum was increased from 10^5 to 10^7, the magnitude of translocation increased and was higher in the monolayers challenged with 10^7 than with 10^5. At an inoculum of 10^2 CFUs (colony forming units) translocation (at 20, 40 min) was hardly observed, even in RNAi (10^2 CFUs) group. However, At an inoculum of 10^2 (at 120, 180 min), 10^5 or 10^7 bacteria, we found that RNAi group showed more BT than control group at each time points, especially at 180 min (2.39 ± 0.21 versus 1.91 ± 0.18 at 10^5 CFUs; 4.12 ± 0.31 versus 3.89 ± 0.32 at 10^7 CFUs).

An increase in BT was also observed which reached statistical significance at 10^5 CFUs or at 10^7 CFUs ($p < 0.01$) between RNAi and CON group, except 10^2CFUs at 20, 40 min (Fig. 3A, B and C) ($p > 0.05$). At 20, 40, 120, and 180 min after inoculation, 10^5 CFUs or 10^7 CFUs E. coli, AQP3 knockdown group showed more BT than those in CON or NC group.

3.4. Effect of E. coli on transepithelial electrical resistance

The TEER was measured before inoculum and 180 min post-inoculum. TEER allows the assessment of monolayer integrity and tight-junction function and it did not decrease over the 180-min experiment in any of the group tested (Fig. 4).

3.5. Localization of AQP3 and the tight junction associated proteins

As an subsidiary approach for assessing the localization of the AQP3 and junctional proteins, immunocytochemistry was used, We showed that both AQP3 and junctional proteins were expressed in Caco-2 cell line. As shown in Fig. 5, The AQP3 and Claudin-1 were mainly located the outer of the membrane, Occludin, Claudin-3, Zo-1 protein were mainly distributed in the cytoplasm and cytomembrane.

3.6. Decreased expression of the tight junction proteins in AQP3 knockdown group

Western blot analysis of the tight junction associated proteins, such as Occludin, Claudins and Zo-1. A significant decrease in Claudin-1 and Occludin expression was observed in AQP3 knockdown

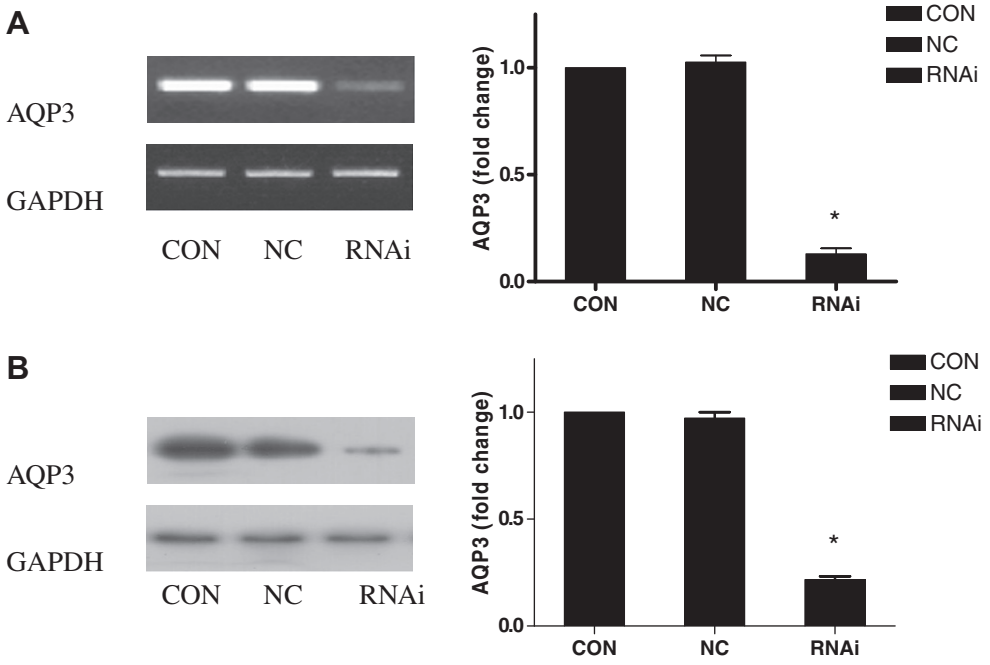

Fig. 1. Decrease in AQP3 expression by RNA interference. Caco-2 cells were cultured and then transfected with shRNA of AQP3 or a scrambled shRNA sequence. AQP3 expression in these cells were analyzed by RT-PCR and western blot. *$p < 0.05$ versus other groups. (A) AQP3 expression was analyzed after transfection by RT-PCR, with GAPDH as loading controls. The figure shows that RNAi induced a specific decrease in AQP3 expression after transfection. (B) Western blot also shows a specific decrease in AQP3 expression after transfection, with GAPDH as loading controls.

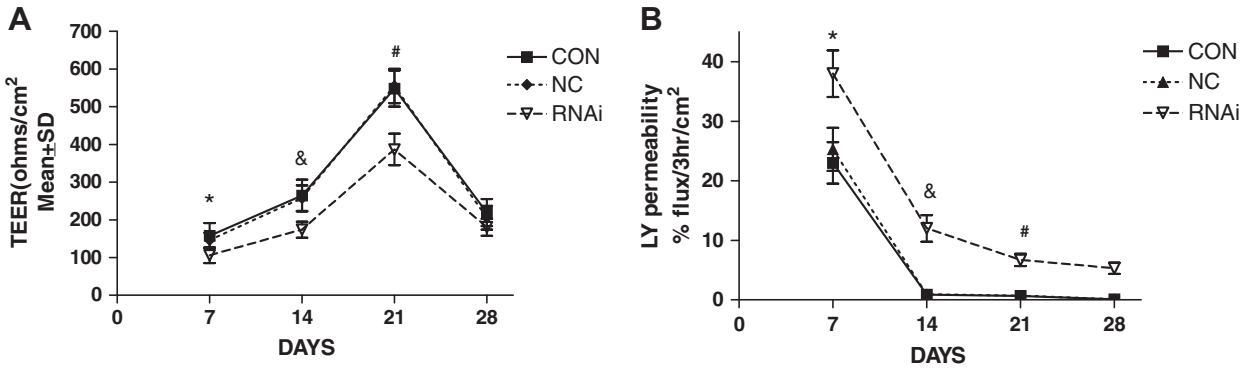

Fig. 2. AQP3 knockdown causes an increase in paracellular permeability ($n = 9$). (A) TEER levels was lower in RNAi group than control group at day 7, 14, 21. (B) Percent of LY crossing Caco-2 monolayer was significant increased in RNAi group than control group at day 7, 14, 21. No significant changes between CON and NC group at day 7, 14, 21($p > 0.05$). $^{*}p < 0.05$ at day 7, group RNAi versus the values in other groups. $^{\&}p < 0.05$ at day 14, group RNAi versus the values in other groups. $^{\#}p < 0.05$ at day 21, group RNAi versus the values in other groups. CON control Caco-2 cells. NC: cells treated with scrambled shRNA. RNAi cells treated with aqp3shRNA.

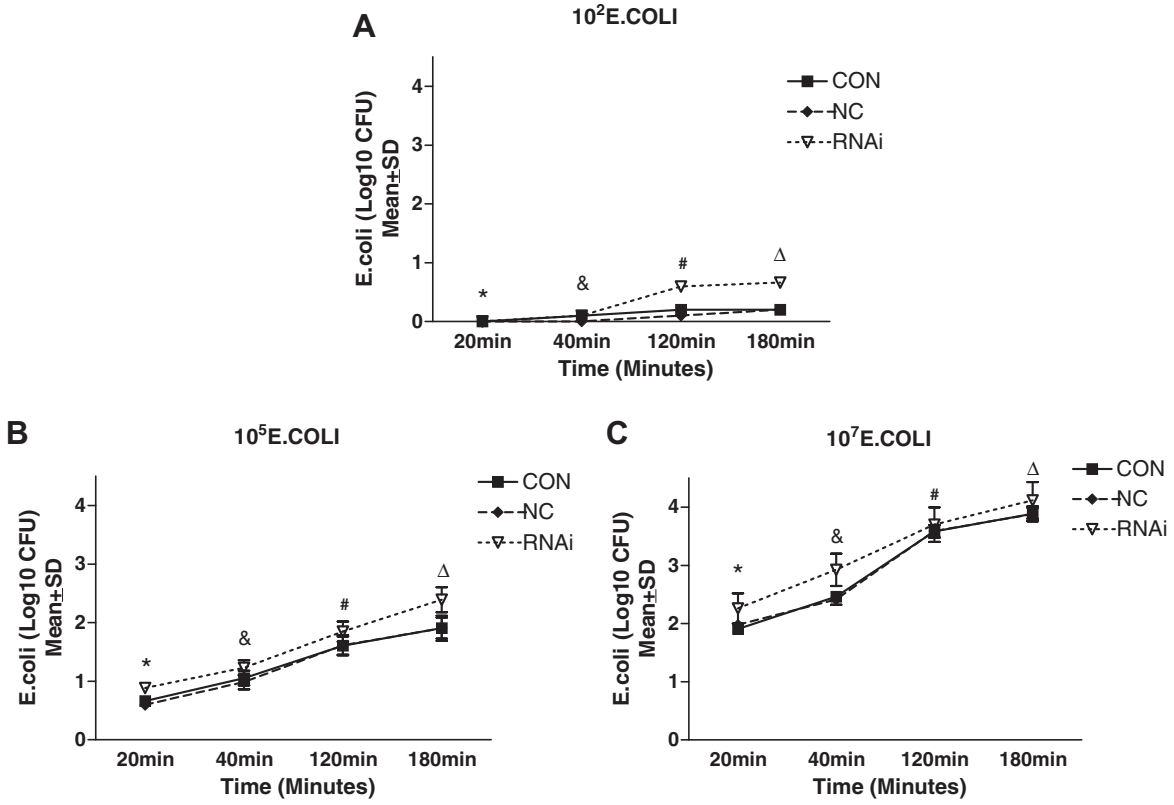

Fig. 3. Effect of time and magnitude of E.coli on bacterial translocation ($n = 9$) Different inoculum (10^2, 10^5 or 10^7) were added to the Caco-2 epithelial monolayer system and the number of translocation bacteria measured at 20, 40, 120, and 180 min after inoculation among CON, NC or RNAi group. The passage of *E. coli* across the Caco-2 monolayer was time and dose dependent. (A) 10^2 CFUs bacteria were added to the Caco-2 epithelial monolayer system and the number of translocation bacteria measured at 20, 40, 120, and 180 min after inoculation. $^{*\&}p > 0.05$ at 20, 40 min respectively, group RNAi versus the values in other groups. $^{\#\Delta}p < 0.05$ at 120,180 min respectively, group RNAi versus the values in other groups. (B) 10^5 CFUs bacteria were added to the Caco-2 epithelial monolayer system and the number of translocation bacteria measured at 20, 40, 120, and 180 min after inoculation. $^{*\&\#}\Delta p < 0.01$ at each time points, group RNAi versus the values in other groups. (C) 10^7 CFUs bacteria were added to the Caco-2 epithelial monolayer system and the number of translocation bacteria measured at 20, 40, 120, and 180 min after inoculation. $^{*\&\#}\Delta p < 0.01$ at each time points, group RNAi versus the values in other groups.

group compare with control group. Expression of other TJs proteins such as Zo-1 did not change significantly (Fig. 6). This is consistent with the observed increase in paracellular permeability.

4. Discussion

Clarification of the physiology of intestinal barrier function has been hampered by the lack of adequate experimental models. Caco-2 cell line, which was extensively used as a model of the human intestinal barrier in vitro [20–22], was selected in this study to understand mechanistically the translocation process. Although the Caco-2 cell line was derived from a human colonic adenocarcinoma cell line, it can mimic normal intestinal epithelium in vitro [23].

AQPs are a family of small (30 kDa/monomer) hydrophobic, integral membrane proteins. AQPs in kidney, gastrointestinal sys-

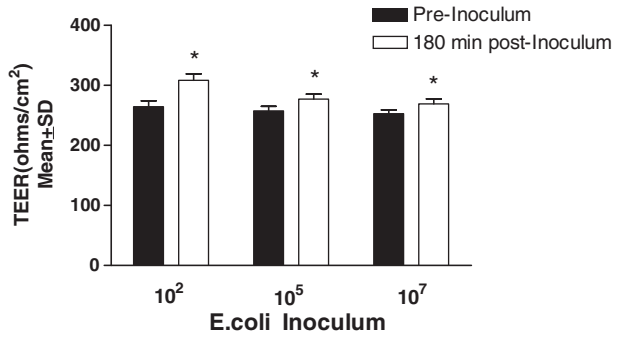

Fig. 4. Comparison of TEERs(mean ± SD) measured before and 180 min after inoculation of the Caco-2 monolayer with *E. coli*. TEER values did not decrease during experimental period. TEER values were higher at end of experimental period than immediate before inoculation ($n = 9$) ($^{*}p < 0.05$).

tem, respiratory apparatus, central nervous system, skin play important roles [24–26]. Papadopoulos MC reported that AQP4-mediated transcellular water movement was crucial in the resolution and formation of vasogenic brain edema [27–28]. Sidhaye overexpressed AQP5 in airway epithelial cells and found that altered AQP5 expression modulates paracellular permeability [29]. Altered ultrastructure of brain microvessels, including impaired tight junctions and blood–brain barrier permeability, were frequently observed in the AQP4 null mice [30]. Kawedia et al. also showed a decrease in the amount of FITC-D transported in the saliva and a decrease in expression of TJs proteins in parotid glands of AQP5−/− mice [31], thus he blocked AQPs by Hg^{2+} and found a 2-fold decrease in tight junction-associated Occludin after Hg^{2+} treatment, the mechanism may be involved in PKA pathway in Hg^{2+}-induced regulation of tight junction permeability [32]. And recently Thiagarajah have proved evidence for involvement of AQP3-mediated colitis in AQP3−/− mice [16]. The transcellular and paracellular routes of water transport have been described in the epithelial tissues. However, the roles of AQPs in the intestinal barrier have not yet been clearly defined. It is not currently known whether these two important pathways interact and whether they compensate for each other or operate coordinately. We reasoned that by measuring TEER and BT, we could directly test the hypothesis that decreasing transcellular water transport could affect paracellular water transport, thus impair the intestinal barrier, leading translocation of intestinal bacteria and endotoxin.

AQP3 localizes to epithelial cells in the human small intestine and colon [33]. To determine whether the functional changes in

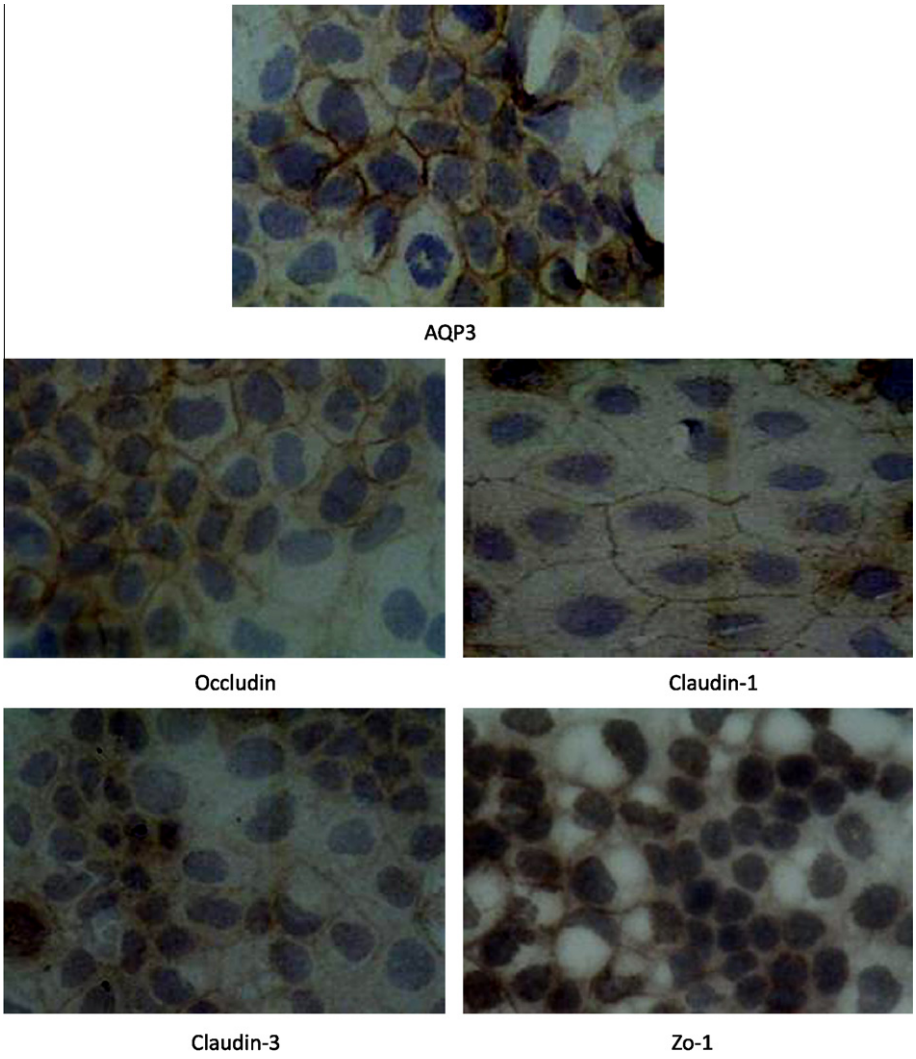

Fig. 5. Localization of AQP3 and the tight junction associated proteins (40×) Immunocytochemistry of the cultured Caco-2 cells showed AQP3 and the tight junction associated proteins expression in their cytoplasm or cytomembrane. Images shown were representative of at least five regions observed on the same slide, and two different sections were analyzed for each condition. Results were based on a double-blinded experiment.

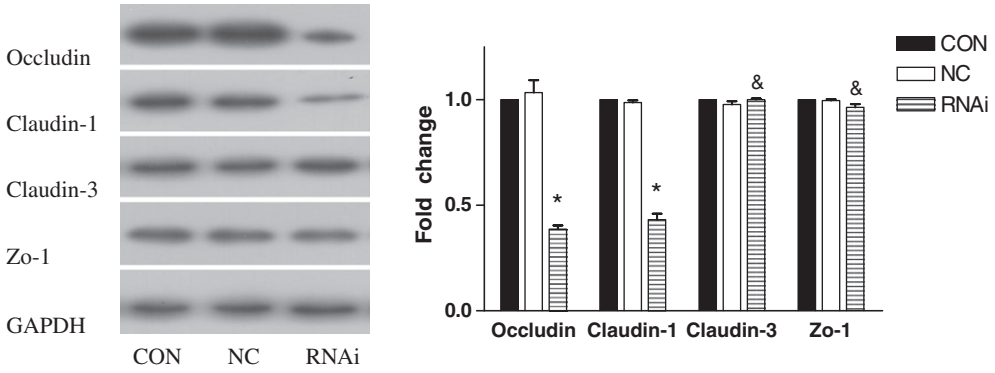

Fig. 6. Western blots. AQP3 knockdown altered the expression of the tight junction associated proteins. A significant decrease in Occludin and Claudin-1 expression was observed in AQP3 knockdown group compare with control group, with GAPDH as loading controls. $^{*}p < 0.05$ versus respective other groups. $^{\&}p > 0.05$ versus respective other groups.

paracellular permeability were paralleled by molecular changes in the TJs, we examined the expression levels of known proteins in the TJs, such as Claudins, Occludin, and Zo-1. Immunocytochemistry showed that junctional proteins were mainly distributed in the cytoplasm and cytomembrane. Of interest, the magnitude of increase in permeability was positively correlated with the decreased expressing of Claudin-1 and Occludin, but not Zo-1 or Claudin-3. Our observation, in vitro, prove that the transcellular and paracellular routes of water transport act in conjunction, which is consistent with Kawedia et al. [31] and Fischbarg [34].

To further define the effect of bacteria translocation and endotoxin after AQPs were knocked down. We have developed an in vitro cultured epithelial monolayer system, utilizing the Caco-2 cell line, in order to investigate the mechanisms of BT. Since Caco-2 Cells forms an impermeable, polarized monolayer and the microvilli in the Caco-2 cell line are well developed, they are chosen in the study. Based on LY permeability results and TEER, it appears that the Caco-2 cells require approximately 14–21 days of culture before form a relatively tight monolayer. After 21 days of cultured the TEER significantly increased to 548 ± 48 ohms/cm^2 and remained impermeable to LY. However, knockdown of AQP3 in Caco-2 cell line, TEER is maintained at a low level and has higher permeability of LY in 7, 14, 21, 28 days compared with the control group, indicating that knockdown of AQP3 (a transcellular protein) impair paracellular transport.

The intestine can absorb and secrete considerable amounts of water even against osmotic gradients, thus helping to maintain the body fluid balance under physiologic condition. Failure of intestinal barrier function resulting in the systemic spread of gut-associated bacteria has been termed BT. Therefore, the subsequent BT studies were carried out with Caco-2 cells in order to make clear the correlation between intestinal barrier damage (AQP3 knockdown) and BT. The results showed that the BT in Caco-2 cell model was time and dose dependent, the magnitude of translocation increased and was higher in the monolayers challenged with 10^7 than with 10^5 or 10^2. As we expected, inoculum of *E. coli* (10^5 or 10^7 CFUs) translocated across the monolayer were greater in AQP3 knockdown group than control group after 180-min experiment. However, inoculum of *E. coli* (10^2 CFUs) did not show significant difference at 20, 40 min. We thought that this was mainly due to the little inoculum of bacteria added to the apical chamber. These results were consistent with western blot. All of these dates demonstrated that knockdown of AQP3 enhances paracellular permeability via an opening of the tight junction complex. Although further studies will be required to elucidate the specific mechanisms, our preliminary results suggest that PKC inhibitor may reverse the process (date not show).

In summary, our results support a novel function of AQP3 in intestinal barrier function, identifying AQP3 as a novel therapeutic target. Further work is needed to investigate the underlying mechanism and the possibility of modulation of AQP3 expression in therapy for diseases, such as inflammatory bowel disease (IBD).

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References

[1] Swank, G.M. and Deitch, E.A. (1996) Role of the gut in multiple organ failure: bacterial translocation and permeability changes. World J. Surg. 20, 411–417.
[2] Moore, F.A. (1999) The role of the gastrointestinal tract in postinjury multiple organ failure. Am. J. Surg. 178, 449–453.
[3] Doig, C.J., Sutherland, L.R., Sandham, J.D., Fick, G.H., Verhoef, M. and Meddings, J.B. (1998) Increased intestinal permeability is associated with the development of multiple organ dysfunction syndrome in critically ill ICU patients. Am. J. Respir. Crit. Care Med. 158, 444–451.
[4] Hang, C.H., Shi, J.X., Li, J.S., Wu, W. and Yin, H.X. (2003) Alterations of intestinal mucosa structure and barrier function following traumatic brain injury in rats. World J. Gastroenterol. 9, 2776–2781.
[5] Turner, J.R. (2009) Intestinal mucosal barrier function in health and disease. Nat. Rev. Immunol. 9, 799–809.
[6] Ma, T. and Verkman, A.S. (1999) Aquaporin water channels in gastrointestinal physiology. J. Physiol. 517 (Pt 2), 317–326.
[7] Zeuthen, T. (2002) General models for water transport across leaky epithelia. Int. Rev. Cytol. 215, 285–317.
[8] Agre, P. and Kozono, D. (2003) Aquaporin water channels: molecular mechanisms for human diseases. FEBS Lett. 555, 72–78.
[9] Cao, C., Sun, Y., Healey, S., Bi, Z., Hu, G., Wan, S., Kouttab, N., Chu, W. and Wan, Y. (2006) EGFR-mediated expression of aquaporin-3 is involved in human skin fibroblast migration. Biochem. J. 400, 225–234.
[10] Shin, K., Fogg, V.C. and Margolis, B. (2006) Tight junctions and cell polarity. Annu. Rev. Cell. Dev. Biol. 22, 207–235.
[11] Tsukita, S. and Furuse, M. (1999) Occludin and claudins in tight-junction strands: leading or supporting players? Trends Cell. Biol. 9, 268–273.
[12] Elkouby-Naor, L. and Ben-Yosef, T. (2010) Functions of claudin tight junction proteins and their complex interactions in various physiological systems. Int. Rev. Cell. Mol. Biol. 279, 1–32.
[13] Hossain, Z. and Hirata, T. (2008) Molecular mechanism of intestinal permeability: interaction at tight junctions. Mol. Biosyst. 4, 1181–1185.
[14] Matsuzaki, T., Tajika, Y., Ablimit, A., Aoki, T., Hagiwara, H. and Takata, K. (2004) Aquaporins in the digestive system. Med. Electron. Microsc. 37, 71–80.
[15] Hara, M. and Verkman, A.S. (2003) Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice. Proc. Natl. Acad. Sci. USA 100, 7360–7365.
[16] Thiagarajah, J.R., Zhao, D. and Verkman, A.S. (2007) Impaired enterocyte proliferation in aquaporin-3 deficiency in mouse models of colitis. Gut 56, 1529–1535.
[17] Gork, A.S., Usui, N., Ceriati, E., Drongowski, R.A., Epstein, M.D., Coran, A.G. and Harmon, C.M. (1999) The effect of mucin on bacterial translocation in I-407 fetal and Caco-2 adult enterocyte cultured cell lines. Pediatr. Surg. Int. 15, 155–159.

[18] Clark, E., Hoare, C., Tanianis-Hughes, J., Carlson, G.L. and Warhurst, G. (2005) Interferon gamma induces translocation of commensal *Escherichia coli* across gut epithelial cells via a lipid raft-mediated process. Gastroenterology 128, 1258–1267.
[19] Hidalgo, I.J., Raub, T.J. and Borchardt, R.T. (1989) Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 96, 736–749.
[20] Sambuy, Y., De Angelis, I., Ranaldi, G., Scarino, M.L., Stammati, A. and Zucco, F. (2005) The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. Cell. Biol. Toxicol. 21, 1–26.
[21] Cruz, N., Qi, L., Alvarez, X., Berg, R.D. and Deitch, E.A. (1994) The Caco-2 cell monolayer system as an in vitro model for studying bacterial-enterocyte interactions and bacterial translocation. J. Burn. Care Rehabil. 15, 207–212.
[22] Liu, Z. and Chen, B. (2004) Copper treatment alters the barrier functions of human intestinal Caco-2 cells: involving tight junctions and P-glycoprotein. Hum. Exp. Toxicol. 23, 369–377.
[23] Cruz, N., Alvarez, X., Berg, R.D. and Deitch, E.A. (1994) Bacterial translocation across enterocytes: results of a study of bacterial-enterocyte interactions utilizing Caco-2 cells. Shock 1, 67–72.
[24] Benga, G. (2009) Water channel proteins (later called aquaporins) and relatives: past, present, and future. IUBMB Life 61, 112–133.
[25] Verkman, A.S. (2002) Physiological importance of aquaporin water channels. Ann. Med. 34, 192–200.
[26] Wang, W., Li, C., Summer, S., Falk, S. and Schrier, R.W. (2010) Interaction between vasopressin and angiotensin II in vivo and in vitro: effect on aquaporins and urine concentration. Am. J. Physiol. Renal Physiol. 299, F577–584.
[27] Papadopoulos, M.C., Manley, G.T., Krishna, S. and Verkman, A.S. (2004) Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. FASEB J. 18, 1291–1293.
[28] Papangelou, A., Lewin 3rd, J.J., Mirski, M.A. and Stevens, R.D. (2009) Pharmacologic management of brain edema. Curr. Treat Options Neurol. 11, 64–73.
[29] Sidhaye, V.K., Schweitzer, K.S., Caterina, M.J., Shimoda, L. and King, L.S. (2008) Shear stress regulates aquaporin-5 and airway epithelial barrier function. Proc. Natl. Acad. Sci. USA 105, 3345–3350.
[30] Zhou, J., Kong, H., Hua, X., Xiao, M., Ding, J. and Hu, G. (2008) Altered blood-brain barrier integrity in adult aquaporin-4 knockout mice. Neuroreport 19, 1–5.
[31] Kawedia, J.D., Nieman, M.L., Boivin, G.P., Melvin, J.E., Kikuchi, K., Hand, A.R., Lorenz, J.N. and Menon, A.G. (2007) Interaction between transcellular and paracellular water transport pathways through Aquaporin 5 and the tight junction complex. Proc. Natl. Acad. Sci. USA 104, 3621–3626.
[32] Kawedia, J.D., Jiang, M., Kulkarni, A., Waechter, H.E., Matlin, K.S., Pauletti, G.M. and Menon, A.G. (2008) The protein kinase A pathway contributes to Hg2+-induced alterations in phosphorylation and subcellular distribution of occludin associated with increased tight junction permeability of salivary epithelial cell monolayers. J. Pharmacol. Exp. Ther. 326, 829–837.
[33] Itoh, A., Tsujikawa, T., Fujiyama, Y. and Bamba, T. (2003) Enhancement of aquaporin-3 by vasoactive intestinal polypeptide in a human colonic epithelial cell line. J. Gastroenterol. Hepatol. 18, 203–210.
[34] Fischbarg, J., Diecke, F.P., Iserovich, P. and Rubashkin, A. (2006) The role of the tight junction in paracellular fluid transport across corneal endothelium. Electro-osmosis as a driving force. J. Membr. Biol. 210, 117–130.