Function and Expression of Cystic Fibrosis Transmembrane Conductance Regulator after Small Intestinal Transplantation in Mice

Penghong Song¹, Wenfeng Song¹, Xiaosun Liu², Changhai Jin³, Haiyang Xie¹, Lin Zhou¹, Biguang Tuo³, Shusen Zheng¹*

¹Key Laboratory of Combined Multi-organ Transplantation of Ministry of Public Health, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China, ²Department of Surgery, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China, ³Department of Gastroenterology, Affiliated Hospital of Zunyi Medical College, Zunyi, China

Abstract

The secretion function of intestinal graft is one of the most important factors for successful intestinal transplantation. Cystic fibrosis transmembrane conductance regulator (CFTR) mediates HCO₃⁻ and Cl⁻ secretions in intestinal epithelial cells. In this study, we made investigation on the expression and function of CFTR in an experimental model of murine small intestinal transplantation. Heterotopic intestinal transplantations were performed in syngeneic mice. The mRNA and protein expressions of CFTR were analyzed by real time PCR and western blot. Murine intestinal mucosal HCO₃⁻ and Cl⁻ secretions were examined in vitro in Ussing chambers by the pH stat and short circuit current (Isc) techniques. The results showed that forskolin, an activator of CFTR, stimulated jejunal mucosal epithelial HCO₃⁻ and Cl⁻ secretions in mice, but forskolin-stimulated HCO₃⁻ and Cl⁻ secretions in donor and recipient jejunal mucosae of mice after heterotopic jejunal transplantation were markedly decreased, compared with controls (P<0.001). The mRNA and protein expression levels of CFTR in donor and recipient jejunal mucosae of mice were also markedly lower than those in controls (P<0.001), and the mRNA and protein expression levels of tumor necrosis factor α (TNFα) were markedly increased in donor jejunal mucosae of mice (P<0.001), compared with controls. Further experiments showed that TNFα down-regulated the expression of CFTR mRNA in murine jejunal mucosa. In conclusion, after intestinal transplantation, the function of CFTR was impaired, and its mRNA and protein expressions were down-regulated, which may be induced by TNFα.

Introduction

Intestinal transplantation is currently accepted as a potential therapeutic option for patients with irreversible intestinal failure, including those with short bowel syndrome, who have life-threatening total parental nutrition complications, e.g. total parental nutrition-related liver dysfunction and difficulty of central venous access. In the past 10 years, the outcomes of intestinal transplantation have been improved dramatically, largely resulting from innovative changes in immunosuppression protocols, surgical advances, improved postoperative care, and accumulated experience. However, the intestinal transplantation continues to be one of the more challenging transplants, with a lower survival compared with that of other solid organ transplants, e.g. liver and renal transplantation [1–4].

The secretion and absorption of intestine is the most important physiological function of intestine. Therefore, the secretion and absorption function of intestinal graft is one of the most important factors for successful intestinal transplantation. The studies have demonstrated that intestinal absorptive function has been impaired following small intestinal transplantation [5,6], and the defects in intestinal absorptive function occur even in nonrejecting small intestinal grafts [7,8]. However, intestinal secretion function after intestinal transplantation is poorly understood. Intestinal secretion not only aids digestion and absorption, but also occurs as a result of some pathophysiologic processes. Intestinal secretion results from the active transports of two principal ions, Cl⁻ and HCO₃⁻ [9]. Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-dependent Cl⁻ channel with Cl⁻ and HCO₃⁻ conductance and abundantly expressed in several functionally diverse tissues, such as the pancreas, intestine, kidney, heart, vas deferens, sweat duct and lung [10,11]. In intestinal epithelial cells, CFTR, which mediates Cl⁻ and HCO₃⁻ transports, plays an important role in the regulation of intestinal secretion function [9,12]. In the present study, we made an investigation on the function and expression of CFTR in intestinal graft, which is very important for understanding intestinal physiologic function after...
intestinal transplantation. The process of intestinal transplantation disturbs intestinal physiological function in various ways, including anatomic changes, ischemia–reperfusion injury, acute and chronic graft rejection, and immunosuppressive therapy. In this study, therefore, we used the mode of syngeneic murine heterotopic jejunal transplantation to seek to identify changes in the function and expression of CFTR in the graft, which is exclusive of the confounding effects of pharmacologic immunosuppression, graft rejection, and graft-versus-host reaction.

Materials and Methods

Establishment of Model of Heterotopic Jejunal Transplantation in Mice

Six to ten week male mice with syngeneic C57BL/6 background were used in this study. The mice were purchased from Shanghai Animal Center (Chinese Academy of Science, Shanghai, China) and housed in the experimental animal facility of Zhejiang University under standard care conditions. All animal operative procedure was approved by the Animal Care Committee of Zhejiang University in accordance with the Principles of Laboratory Animal Care (NIH publication 85-23, revised 1985). Murine heterotopic jejunal transplantation was performed according to Zhong et al. [13], with some modifications. Syngeneic C57BL/6 mice were used as donors and recipients. Briefly, after anesthetization by intraperitoneal injection of 50 mg/kg ketamine and 10 mg/kg xylazine, 7 cm donor’s upper portion of jejunum was harvested with the Carrel’s patches of superior mesenteric artery and portal vein which was stored in ice-cold saline until implantation. Using a 10-0 nylon suture, the donor’s Carrel’s patches of superior mesenteric artery and portal vein were anastomosed to the recipient abdominal aorta and inferior vena cava, respectively, yielding end-to-side anastomoses in both cases. The proximal end of the graft was closed with 7-0 silk suture, and the distal end of the graft was exteriorized as a stoma. Recipient mice were sacrificed 2 week after transplantation, and graft and host jejunum were used for experiments.

Ussing Chamber Experiments

After brief narcosis with 100% CO₂, the mice were killed by cervical dislocation. The abdomen was opened by midline incision. The jejunal grafts and host jejunums in the experimental mice and jejunums of normal controls were removed and immediately placed in ice-cold iso-osmolar mannitol and indomethacin (1 mmol/L) solution (to suppress trauma-induced prostaglandin release). The jejunums were opened and stripped of external serosal and muscle layers by sharp dissection in the above-mentioned iso-cold iso-osmolar mannitol and indomethacin solution. Ussing chamber experiments were performed as previously described [14]. Briefly, the jejunal mucosa were mounted between two chambers with an exposed area of 0.196 cm² and placed in an Ussing chamber. Paraffin “O” ring was used to minimize edge damage to the tissue where it was secured between the chamber halves. The mucosal side was bathed with unbuffered HCO₃⁻ free modified Ringer’s solution circulated by a gas lift with 100% O₂ to facilitate the measurement of HCO₃⁻ secretion by pH stat method. The serosal side was bathed with modified buffered Ringer’s solution (pH 7.4) containing 25 mmol/L HCO₃⁻ and gassed with 95% O₂/5% CO₂. Each bath contained 10.0 ml of the respective solution maintained at 37°C by a heated water jacket. Experiments were performed under continuous short-circuit conditions to maintain the electrical potential difference at zero, except for a brief period (<2 seconds) at each time point when the open-circuit potential difference was measured. Luminal pH was maintained at 7.40 by the continuous infusion of 0.5 mmol.L⁻¹ HCl under the automatic control of a pH-stat system (B42 Tirando, pH-Stat Controller, Metrohm). The volume of the titrant infused per unit time was used to quantitate HCO₃⁻ secretion. These measurements were recorded at 5-minute intervals. The rate of luminal HCO₃⁻ secretion is expressed as μmol.cm⁻².h⁻¹. The rate of Cl⁻ secretion was examined by transepithelial short-circuit current (Isc, reported as μEq.cm⁻².h⁻¹), which was measured via an automatic voltage clamp (Voltage-Current Clamp, EVC-1000; World Precision Instruments, USA).

RNA Extraction and Real-time RT-PCR

Total RNA from the mucosae of jejunal graft, host jejunum, and control jejunum was extracted using Trizol Reagent (Invitrogen) according to the manufacturer’s instructions. The concentrations of all RNA samples were determined spectrophotometrically. The cDNA was produced from 2 μg of total RNA using M-MLV reverse transcriptase (Promega) according to the manufacturer’s instructions. Quantitative real-time RT-PCR was performed on a 7500 Fast Real-Time PCR system (Applied Biosystems, USA) using SYBR® Premix Dimer Eraser (Takara Bio, Dalian, China) following the manufacturer’s instruction. All samples were run in triplicate and β-actin was used as an internal control. The expression levels of CFTR, solute carrier family 26 member a3 (Slc26a3), solute carrier family 26 member a6 (Slc26a6), tumor necrosis factor α (TNFα), and interferon γ (IFNγ) mRNA were normalized to that of β-actin and were expressed as a ratio relative to β-actin. The primers were as follows: CFTR forward: AAGGCGGCCCATAATGAGGT, reverse: AGAGCATTCCCGTGATGAC; Slc26a3 forward: GAATCTGTGATCGACTTTCTGAA, reverse: GAGTCCCGAGAACATGTA; Slc26a6 forward: TTGGTTGAGTTGTGATGAC, reverse: ATGTGGCCTGACGACTCTCCTAGC; TNFα forward: AGGGCGATGCTGGTTACCTG, reverse: GACGGCAGAGAGGATGTTG; IFNγ forward: CCTGGCCCTAGCTCTGAG, reverse: GCCATGAGGAGACGCTGGA; β-actin forward: TACAGCCTTACCCACGAC, reverse: TCTCCAGGGCAGAGAGAT.

Western Blot Analysis

The mucosae from jejunal graft, host jejunum, and control jejunum were homogenized in lysis buffer (1% Triton X-100, 10 mmol/L Tris, 100 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% SDS, 0.5% Deoxycholate, 1 mmol/L NaF, 1 mmol/L phenylmethylsulfonyl fluoride, 40 μg of leupeptin/ml, 5 μg of aprotinin/ml, 1 μg of pepstatin/ml) at 4°C. After centrifugation at 14000 rpm for 30 minutes at 4°C, the protein concentrations of supernatants in samples were measured by the BCA protein assay (Pierce, USA). Aliquots of supernatants were used for detecting CFTR, Slc26a3, Slc26a6, TNFα, and IFNγ protein using affinity-purified polyclonal antibodies respectively. β-actin was served as internal control. Protein bands were analyzed with image analysis software. Results were expressed as the ratio relative to β-actin.
Immunohistochemistry Analysis

Immunohistochemical staining was done on the formalin-fixed and paraffin-embedded tissue blocks as previously described [15]. The tissue blocks were cut into 5 μm sections, deparaffinized, and rehydrated. Antigen retrieval was performed in 10 mmol/L citric acid buffer (pH 6.0) in a 750 W microwave for 15 minutes. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 15 minutes. After incubation with anti-TNFα antibody (1:800 dilution, Abcam) overnight at 4°C, the sections were washed in PBS and then incubated with labeled polymer horseradish peroxidase rabbit antibody (Invitrogen) for 1 hour. The sections were rinsed in PBS, incubated with Dako Liquid DAB Large-Volume Substrate-Chromogen System, rinsed gently in distilled water, and counterstained with hematoxylin. Negative controls were also prepared in all assays by replacing anti-TNFα antibody with nonimmune rabbit antiserum. Computer-assisted quantification of the immunostaining was performed as described by Pilette et al [16] with an optical microscope (Olympus) equipped with a charge-coupled device (CCD) camera and image analysis software, using a final magnification of 400 ×. Data were collected from an average of 10 randomly selected areas in a random section (average 10 sections) from each examined tissue. Results were expressed as the mean optical density (measured in arbitrary units), representing the mean intensity of staining in the considered area.

Statistics

All results are expressed as mean ± SEM. ΔHCO3- and ΔI(HCO3-) both refer to stimulated peak responses minus basal levels. Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls's post-hoc test or, when appropriate, by the two-tailed student t tests. P<0.05 was considered statistically significant.

Results

Expressions of mRNA and Protein of CFTR after Jejunal Transplantation

Intestinal secretion results from the active transports of two principal ions, Cl− and HCO3-. It is well known that CFTR, which mediates intestinal epithelial Cl− and HCO3- transports, plays an important role in the regulation of intestinal secretion function. Therefore, we firstly examined the mRNA and protein expressions of CFTR in both donor and recipient jejunal mucosae after heterotopic jejunal transplantation. As shown in Figure 1, the mRNA and protein expression levels of CFTR in both donor and recipient jejunal mucosae were decreased after heterotopic jejunal transplantation, compared with controls (P<0.001). In addition to CFTR, Slc26a3 and Slc26a6 are two major Cl−/HCO3- exchangers and also regulate Cl− and HCO3- transports of intestinal epithelial cells [17]. We further examined the mRNA and protein expressions of Slc26a3 and Slc26a6 in donor and recipient jejunal mucosae after heterotopic jejunal transplantation. As shown in Figure 2 and Figure 3, the mRNA and protein expression levels of both Slc26a3 and Slc26a6 in donor and recipient jejunal mucosae were not significantly altered after heterotopic jejunal transplantation, compared with controls (P>0.05). These results indicated that the mRNA and protein expressions of CFTR in intestinal epithelial cells, but not Slc26a3 and Slc26a6, are down-regulated after intestinal transplantation.

CFTR Function after Jejunal Transplantation

The previous studies have demonstrated that forskolin is a CFTR activator in intestinal mucosal epithelial cells [18,19]. We examined the alteration of CFTR function after jejunal transplantation through the application of forskolin. As shown in Figure 4, forskolin-stimulated jejunal mucosal epithelial HCO3- secretion.
secretion and Isc were markedly decreased in both donor and recipient jejunums after heterotopic jejunal transplantation, compared with controls. Forskolin-stimulated jejunal mucosal epithelial net peak HCO₃⁻ secretion and Isc were decreased by 50.35% and 56.83% in donor jejunum (P<0.001) and by 47.97% and 51.66% in recipient jejunum (P<0.001), respectively. The results indicated that CFTR function is impaired in both donor and recipient jejunums after heterotopic jejunal transplantation.

Figure 2. Expressions of mRNA (A) and protein (B) of Slc26a3 in both donor and recipient jejunal mucosae. Upper panel in B is a representative blot graph of Slc26a3 protein. Lane 1: Control; Lane 2: Donor; Lane 3: Recipient. The results are expressed as a ratio to β-actin. Values are mean ± SEM and n = 6 in each series. The mRNA and protein expression levels of Slc26a3 in both donor and recipient jejunal mucosae were not altered significantly, compared with controls. *P<0.05.
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Figure 3. Expressions of mRNA (A) and protein (B) of Slc26a6 in both donor and recipient jejunal mucosae. Upper panel in B is a representative blot graph of Slc26a6 protein. Lane 1: Control; Lane 2: Donor; Lane 3: Recipient. The results are expressed as a ratio to β-actin. Values are mean ± SEM and n = 6 in each series. The mRNA and protein expression levels of Slc26a6 in both donor and recipient jejunal mucosae were not altered significantly, compared with controls. *P<0.05.
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Expressions of mRNA and Protein of Cytokines IFNγ and TNFα after Jejunal Transplantation

The previous studies have shown that cytokines, IFNγ and TNFα, regulated the expression of CFTR in epithelial cells [20-22]. Therefore, we examined the mRNA and protein expressions of cytokines IFNγ and TNFα in both donor and recipient jejunal mucosae after heterotopic jejunal transplantation. The results showed that the mRNA and protein expression levels of IFNγ in both donor and recipient jejunal mucosae were not significantly altered after heterotopic jejunal transplantation, compared with controls (P > 0.05, Figure 5). The mRNA and protein expression levels of TNFα in recipient jejunal mucosa were not significantly altered either, compared with controls (P > 0.05), but the mRNA and protein expression levels of TNFα in donor jejunal mucosa was markedly increased, compared with controls (P < 0.001) (Figure 6). We further examined the expression and location of TNFα in jejunal mucosa by immunohistochemistry. The results showed that TNFα was expressed in the monocytes of intestinal mucosa and there were not the expressions of TNFα in both villous and cryptal epithelial cells of intestinal mucosa, and further confirmed the increased expression of TNFα in jejunal mucosa of donor after transplantation compared with control (Figure 7) (P < 0.001).

Effect of TNFα on the mRNA Expression of CFTR in Jejunal Mucosa

We further examined the effect of TNFα on the mRNA expression of CFTR in jejunal mucosa through the experiments in vitro. As shown in Figure 8, the mRNA expression level of CFTR in jejunal mucosa was markedly decreased at 1 hour after the incubation of murine jejunal mucosa with TNFα, compared with control (P < 0.05), but not time-dependent. The results indicated that TNFα may down-regulate the mRNA expression of CFTR in jejunal mucosa.
Figure 5. Expressions of mRNA (A) and protein (B) of IFNγ in both donor and recipient jejunal mucosae. Upper panel in B is a representative blot graph of IFNγ protein. Lane 1: Control; Lane 2: Donor; Lane 3: Recipient. The results are expressed as a ratio to β-actin. Values are mean ± SEM and n = 6 in each series. The mRNA and protein expression levels of IFNγ in both donor and recipient jejunal mucosae were not significantly altered, compared with controls. *P < 0.05.
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Figure 6. Expressions of mRNA (A) and protein (B) of TNFα in both donor and recipient jejunal mucosae. Upper panel in B is a representative blot graph of TNFα protein. Lane 1: Control; Lane 2: Donor; Lane 3: Recipient. The results are expressed as a ratio to β-actin. Values are mean ± SEM and n = 6 in each series. The mRNA and protein expression levels of TNFα in recipient jejunal mucosa were not significantly altered, compared with controls. However, the mRNA and protein expression levels of TNFα in donor jejunal mucosa were markedly higher than those in controls. *P < 0.05; **P < 0.001.
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Discussion

In the present study, our results demonstrated that after intestinal transplantation, the function of CFTR was impaired, and its mRNA and protein expressions were down-regulated, which may be induced by TNFα.

Although the outcomes of intestinal transplantation have been improved dramatically in the past 10 years, largely through advances in immunosuppression protocols, improved surgical technique and postoperative care, and accumulated experience, the clinical results are not so satisfactory yet, compared with those of other solid organ transplants, e.g. liver and renal transplantation. The intestine functions as both a secretory and an absorptive organ and is in a dynamic state of secretion and absorption, capable of transporting very large volumes of fluid and electrolytes, which is necessary for digestion and physiologic homeostasis. Therefore, the secretion and absorption function of intestinal graft is a factor that possibly contributes to the clinical success of intestinal transplantation. CFTR is a cAMP-activated epithelial Cl⁻ channel with Cl⁻ and HCO₃⁻ conductance and abundantly expressed in several functionally diverse tissues. In addition to its involvement in epithelial Cl⁻ and HCO₃⁻ secretions, CFTR also regulates other plasma membrane proteins, including the outwardly rectifying Cl⁻ channels, epithelial Na⁺ channels, K⁺ channels, ATP-release mechanisms, anion exchangers, Na⁺-HCO₃⁻ cotransporters, and aquaporin water channels [23-25]. Thus CFTR might be central in determining transepithelial salt transport, fluid flow, and intracellular ion concentrations. In intestinal epithelial cells, CFTR plays an important role in the regulation of fluid, Cl⁻, and HCO₃⁻ transports [9,12]. Intestinal disease in cystic fibrosis is primarily targeted to the small intestine and is characterized by defective alkalization of secretions in the proximal small intestine, luminal obstruction by thick mucoid secretions, and malabsorption [26]. However, little is known of the function and expression of CFTR after intestinal transplantation. The previous studies have shown that forskolin stimulates...
intestinal mucosal epithelial HCO$_3^-$ and Cl$^-$ secretions and the deletion of CFTR gene completely abolished forskolin-stimulated intestinal mucosal epithelial HCO$_3^-$ and Cl$^-$ secretions [18,19], strongly demonstrating forskolin induces intestinal mucosal epithelial HCO$_3^-$ and Cl$^-$ secretions through CFTR. In this study, we found that the mRNA and protein expression levels of CFTR, but not Cl$^-$/HCO$_3^-$ exchanges Slc26a3 and Slc26a6, were markedly decreased in both donor and recipient jejunal mucosae after heterotopic jejunal transplantation compared with controls. The further examination found that forskolin-stimulated jejunal mucosal epithelial HCO$_3^-$ and Cl$^-$ secretions were markedly decreased in both donor and recipient jejunal mucosae compared with controls. The results indicated that the CFTR function of intestinal graft is impaired and its mRNA and protein expressions are down-regulated after intestinal transplantation.

What is responsible for the alterations of function and expression of CFTR after intestinal transplantation? The previous studies have shown that TNF$\alpha$ down-regulated CFTR mRNA expression in HT-29 cells, a colon epithelium-derived tumor cell line, in a dose- and time-dependent fashion [22]. IFN$\gamma$, but not IFN$\alpha$ or IFN$\beta$, down-regulated CFTR mRNA levels in two colon- derived epithelial cell lines, HT-29 and T84 cells, in a time- and concentration-dependent manner [20]. And TNF$\alpha$ and IFN$\gamma$ synergistically decreased CFTR mRNA expressions in HT-29 and T84 cells [27]. In addition, TNF$\alpha$ and IFN$\gamma$ decreased agonist-stimulated CFTR-mediated Cl$^-$ secretion in T84 cells [27,28]. In this study, therefore, we first examined the mRNA and protein expressions of TNF$\alpha$ and IFN$\gamma$ in donor and recipient jejunal mucosae after heterotopic jejunal transplantation. The results showed that the mRNA and protein expression levels of IFN$\gamma$ in donor and recipient jejunal mucosae were not altered significantly compared with controls, and the mRNA and protein expression levels of TNF$\alpha$ in recipient jejunal mucosae was not altered significantly either. However, the mRNA and protein expression levels of TNF$\alpha$ in donor jejunal mucosa were markedly higher than those in controls. Immunohistochemical results also showed an enhanced expression of TNF$\alpha$ in donor jejum. The results indicated that it is possible that TNF$\alpha$ induces the alterations of function and expression of CFTR. Our results showed that the alterations of CFTR expression and function occurred in both donor and recipient jejunal mucosae, but the alteration of TNF$\alpha$ expression only occurred in the donor jejum, indicating that TNF$\alpha$ might exert its effect on the recipient jejum through blood circulation in addition to its local action on the donor jejum. The further experiments showed that the incubation of jejunal mucosa with TNF$\alpha$ in vitro decreased the mRNA expression level of CFTR in jejunal mucosa. Taken together, these results indicate that TNF$\alpha$ induces the alterations of function and expression of intestinal CFTR after intestinal transplantation.

TNF$\alpha$ is a potent pro-inflammatory cytokine that regulates essential biological functions (e.g., cell differentiation, proliferation, survival, apoptosis) and a broad spectrum of responses to stress and injury, and plays a critical role in the pathogenesis of chronic inflammatory diseases [29,30]. It is primarily produced by immune cells such as monocytes and macrophages, but it can also be released by many other cell types, including acinar cells. The studies in the experiments in vitro showed that human colonic epithelial cells might produce a wide range of proinflammatory cytokines, including TNF-$\alpha$, in response to invasive microbial pathogens [31,32]. In this study, our immunohistochemical results showed that TNF$\alpha$ was located in the monocytes of intestinal mucosa, and there were not the expressions of TNF$\alpha$ in both villous and cryptal epithelial cells of intestinal mucosa. The results indicate that TNF$\alpha$ is primarily produced by the monocytes of intestinal mucosa after the intestinal transplantation.

What induced the increase of TNF$\alpha$ in the mucosa of the intestinal graft? In this study, we used the mode of syngeneic murine heterotopic jejunal transplantation, which is exclusive of the effects of graft rejection and graft-versus-host reaction. In addition, the expression of TNF$\alpha$ was only enhanced in the donor jejunal mucosa, not in the recipient jejunal mucosa, after the heterotopic jejunal transplantation, indicating that it is possible
that anatomic changes, including transection of the intestinal wall along with intrinsic neurons, complete extrinsic autonomic denervation, and the disruption of lymphatic drainage, or ischemia-reperfusion injury induced the increase of TNFα in the mucosa of the intestinal graft. Although infection and endotoxemia are potent stimulants of TNFα production, the studies also found that intestinal ischemia-reperfusion increased TNFα levels in the serum and intestinal tissue of rat [33-35]. In addition, enteric nervous system also plays an important role in the regulation of intestinal immune function. The interactions between the enteric nervous system and local immunocytes are responsible for many functional changes, including motility and secretion. Several neuropeptides, such as tachykinins, vasoactive intestinal peptide, somatostatin, and opioids, are involved in both intrinsic and extrinsic innervation but can also affect the release of cytokines and proinflammatory mediators. On the other hand, proinflammatory mediators, such as eicosanoids and cytokines, may activate intrinsic neurons directly or stimulate extrinsic neurons indirectly, releasing neuropeptides which act on intrinsic neurons, smooth muscle cells, or enterocytes [36,37]. In several models of experimental inflammation, intrinsic denervation as well as destruction of sensory C fibres affected the local immune reactions. The functional ablation of sensory neurons by capsaicin pretreatment worsened colitis in rabbit [38]. The vagotomy significantly increased the TNF-α levels in serum and colonic tissue in mice [39,40]. These studies indicate that extrinsic and intrinsic denervations might affect the release of TNF-α in the monocytes of intestinal mucosa.

In conclusion, our study demonstrates that intestinal transplantation impairs the CFTR function and down-regulates the expression of CFTR in intestinal mucosal epithelial cells, even in non-rejecting small intestinal graft. It also implicates that the examination of CFTR mRNA in biopsy specimens following intestinal transplantation may provide useful information for evaluating intestinal graft function.

Author Contributions

Conceived and designed the experiments: PS SZ. Performed the experiments: PS WS XL CJ HX LZ. Analyzed the data: PS WS XL CJ HX LZ BT SZ. Contributed reagents/materials/analysis tools: PS. Wrote the paper: PS BT SZ.

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Author Contributions

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