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Short Communication

Effects of Epidermal Growth Factor on ASCT2 Expression in IPEC-J2 Cells Challenged by Lipopolysaccharide

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Authors' Contribution
XT and RF designed and performed the experiments. XT analyzed the data, wrote this article. KX provided the financial support for this study.

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ABSTRACT

The aim of this study was to investigate the effect of epidermal growth factor (EGF) on ASCT2 expression in IPEC-J2 cells challenged by lipopolysaccharide (LPS). Cells were treated with: (1) EGF (0 ng / mL) + LPS (0 μg / mL) (Control group), (2) EGF (100 ng / mL) + LPS (0 μg / mL) (EGF group), (3) EGF (0 ng / mL) + LPS (1 μg / mL) (LPS group), and (4) EGF (100 ng / mL) + LPS (1 μg / mL) (EGF+LPS group) for 24 h. The gene expression of glutamine transport protein Na+-dependent neutral amino acid transporter (ASCT2) and protein expression of ASCT2 were measured. The results showed that LPS significantly ($P<0.05$) decreased the gene and protein expression of ASCT2 in IPEC-J2 cells. EGF significantly ($P<0.05$) promoted the gene and protein expression of ASCT2. EGF plus LPS group had a higher ($P < 0.05$) gene and protein expression of ASCT2 than that LPS treated group, although significantly ($P < 0.05$) lower than EGF treated group, but had no difference between the Control group. It can speculate that EGF promoted the absorption of glutamine through activating ASCT2 expression in injured intestinal epithelial cells.

Glutamine (Gln) is one of the most abundant amino acids in the body, which serves important roles in maintaining intestinal integrity and healthy gastrointestinal function (Huang *et al*., 2007; Pochini *et al*., 2014; Yi *et al*., 2015). Gln is the main source of energy for intestinal epithelial cells, and the transported of Gln across intestinal epithelium is depend on specificity carriers, in which a Na+-dependent neutral amino acid transporter, Alanine-Serine-Cysteine Transporter 2 (ASCT2) is the most important Gln transporter (Ray *et al*., 2005; Huang *et al*., 2007; Avissar *et al*., 2008). Since Gln plays a central role in cell protein and energy metabolism, the regulation of ASCT2 expression to mediate Gln absorption across intestinal epithelium is particularly important.

Epidermal growth factor (EGF) is a small mitogenic polypeptide comprising 53 amino acid residues, which had established as a cytoprotective peptide that plays pivotal roles in epithelial cell homeostasis maintenance and nutrients transport in the small intestine (Tang *et al*., 2016, 2018; Wang *et al*., 2019). *In vivo* and *in vitro* studies have shown that EGF can promote the absorption of Gln in intestinal epithelial cells by increasing ASCT2 expression (Huang *et al*., 2007; Avissar *et al*., 2008). However, the studies on the effects of EGF on absorption of Gln through activating ASCT2 expression in piglets under stress conditions have rarely been reported. Therefore, the present study used lipopolysaccharide (LPS) to establish a cell injury model (Tang *et al*., 2018). Here, we investigate the effect of EGF on ASCT2 expression in porcine intestinal epithelial cells (IPEC-J2) cells under stress condition.

Materials and methods

IPEC-J2 were obtained from Institute of Subtropical Agriculture, Chinese Academy of Science (Changsha, China) and cultured in DMEM / F12 medium (GE Healthcare life sciences, South Logan, Utah, USA) containing 10% FBS (GIBCO, Carlsbad, CA, USA), 1% antibiotics (Penicillin-Streptomycin) (GIBCO, Carlsbad, CA, USA), and grown in a humidified incubator at 37°C with 5 % CO_2 and 95 % air as previously described (Tang *et al*., 2018). After 80% of fusion, cells were digested with 0.25% trypsin-EDTA (GIBCO, Carlsbad, CA, USA).

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The cells were seeded in 6-well plates (1×10^5 / well) and cultured in DMEM / F12 with 10% FBS and 1% antibiotics for 24 h and then treated with (1) EGF (0 ng / mL) + LPS (0 μg / mL) (Control group), (2) EGF (100 ng / mL) + LPS (0 μg / mL) (EGF group), (3) EGF (0 ng / mL) + LPS (1 μg / mL) (LPS group), and (4) EGF (100 ng / mL) + LPS (1 μg / mL) (EGF+LPS group) for 24 h. EGF was purchased from Peprotech (Rocky Hill, NJ, USA). LPS was purchased from Sigma-Aldrich (Saint Louis, MO, USA). The EGF and LPS concentrations were adopted according to Tang *et al*. (2018).

Total cell RNA was extracted and purified using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) for Real-time PCR analysis of *ASCT2* mRNA. The PCR procedure was performed as described previously (Tang *et al*., 2018). Briefly, the total RNA was reverse transcribed to cDNA. Then the RT-PCR was performed using the SYBR® Premix Ex Taq™ (Takara, Dalian, China) on an Applied Biosystems 7500 Fast Real-Time PCR System (Foster City, CA, USA). All PCRs were performed in triplicate on a 96-well RT-PCR plate under the following conditions: 95°C for 10 min followed by 40 cycles of 95°C for 15s, 60°C for 30s and 72°C for 60s. The primers used in this study were synthesized by Sangon Biotech (Shanghai, China), which listed in Table I.

Table I. Primers used for quantitative reverse transcription PCR.

Genes	Primers squence	Product length
β-actin	F: 5'- GTTCGAGACCTTCAACACCCC -3' R:5'- CCGGCCAGCCAGGTCCAGA -3'	181 bp
ASCT2	F:5'- GATCCCCATTGGCACCGAGA -3' R:5'- CATGACACCAGCACCATCGTT -3'	159 bp

Total protein was extracted using RIPA Lysis Buffer R2220 (containing 1% PMSF) (Solarbio, Beijing, China) for Western blot analysis of ASCT2 prorein. Western blot was performed as described previously (Tang *et al*., 2018). The antibody information was listed in Table II. Briefly, loaded the protein samples for SDS-PAGE and subsequently transferred to PVDF membrane. The membrane was blocked with PBST buffer containing 5% skim-milk for 1 h at room temperature followed by overnight hybridization at 4°C with the indicated primary anti-bodies of anti-ASCT2 and anti-β-actin. After incubation with secondary antibody, HRP goat anti-rabbit IgG (Proteintech, Rosemont, IL, USA) for 1 h, signals were detected using enhanced chemiluminescence kits (ECL-Plus, Thermo, Waltham, MA, USA), and then scanned for detection of fluorescence using the BioRad gel detection system. Experiments were performed in triplicate.

For data analysis, all data were expressed as mean ± standard deviation (SD). Data were performed by one-way ANOVA procedure of SPSS 21.0 software (SPSS, Inc., Chicago, IL, USA). Differences among treatment mean were determined using Duncan's multiple comparison test. $P < 0.05$ was considered significant.

Table II. Antibodies message used for western blot.

Antibodies	Catalog number	Source	Dilu-tion	Company
anti-ASCT2	20350-1-AP	Rabbit	1:8000	Proteintech, USA
anti-β-actin	60008-1-Ig	Mouse	1:4000	Proteintech, USA

Results and discussion

Gln is an important nutrient in the intestinal repair process, since it directly provides energy to the epithelial cells (Pochini *et al*., 2014; Yi *et al*., 2015). Since ASCT2 is the major Gln transporter in epithelial cells (Ray *et al*., 2005; Huang *et al*., 2007; Avissar *et al*., 2008), the regulation of ASCT2 expression to mediate Gln absorption across intestinal epithelium is particularly important for intestinal development and intestinal repair. The present study has investigated the effect of epidermal growth factor (EGF) on ASCT2 expression in IPEC-J2 cells challenged by LPS. The gene expression of *ASCT2* mRNA is presented in Figure 1, and the protein expression of ASCT2 is presented in Figure 2. The results showed that IPEC-J2 cells challenged with LPS, the gene and protein expression of ASCT2 both decreased significantly ($P<0.05$) compared with control group. EGF significantly ($P<0.05$) promoted the gene and protein expression of ASCT2. EGF plus LPS group had a higher ($P < 0.05$) gene and protein expression of ASCT2 than that LPS treated group, although significantly ($P < 0.05$) lower than EGF treated group, but had no difference compared with the control group. It indicated that EGF had a protective effect on IPEC-J2 cells challenged by LPS through promoting ASCT2 expression.

Small intestine is the main site of the nutrient absorption and transport in the digestive tract, the integrity of intestinal mucosa is very important to maintain the digestion and absorption function of animals (Tang *et al*., 2016). Disruption of the intestinal epithelial homeostasis would cause a decline in the intestinal absorption function. Previous studies had demonstrated that LPS has a strong cytotoxicity which can induced serious oxidative damage (Talavera *et al*., 2017; Tang *et al*., 2018), immunological stress (Zhou *et al*., 2017) and barrier function damage (Yang *et al*., 2015). The intestinal injury induced by LPS

can lead to intestinal nutrient absorption disorders, which is demonstrated by the present study that LPS significantly inhibited ASCT2 expression.

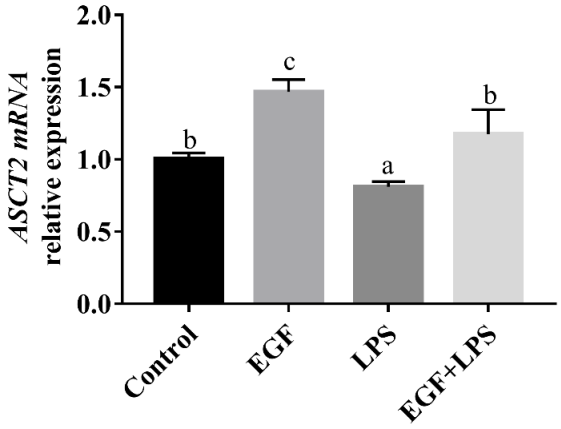

Fig. 1. Effects of EGF on *ASCT2* mRNA expression in IPEC-J2 cells challenged by LPS. Densitometric values were normalized to *β-actin* and expressed as a relative level to control values. Data are presented as mean ± SD, n=3, a, b, c means different lowercase letters indicate significant differences ($P < 0.05$).

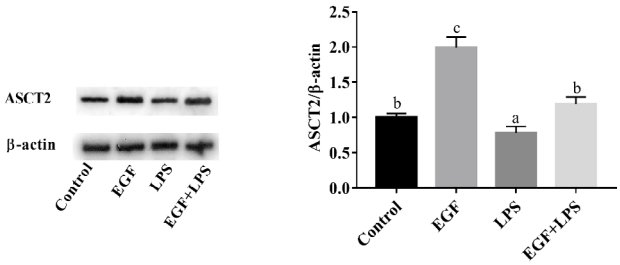

Fig. 2. Effects of EGF on ASCT2 protein expression in IPEC-J2 cells challenged by LPS. Densitometric values were normalized to β-actin and expressed as a relative level to control values. Data are presented as mean ± SD, n=3, a, b, c means different lowercase letters indicate significant differences ($P < 0.05$).

EGF had established as a trophic factor for the intestinal mucosa homeostasis (Bedford *et al*., 2015; Xu *et al*., 2015; Tang *et al*., 2018), which is benefit for nutrients absorption. Previous studies have demonstrated that EGF could promote nutrients absorption, including Na^+ (Ruhul *et al*., 2011), Cl^- (O'Mahony *et al*., 2008), Ma^{2+} (Ledeganck *et al*., 2013), glucose (Wang *et al*., 2019), peptide (Xu *et al*., 2015) and Gln (Ray *et al*., 2005; Huang *et al*., 2007; Avissar *et al*., 2008). Huang *et al*. (2007) reported that EGF could reverse ischemic injured ASCT2 down-expression in human intestinal epithelial cells. It indicated that EGF could through increasing the absorption of Gln repair injured intestinal epithelial cells. However, whether EGF has a positive effect on Gln absorption in injured porcine intestinal epithelial cells has not yet been reported. The present study used IPEC-J2 as a cell model and challenged by LPS to investigate the effect of EGF on ASCT2 expression in injured IPEC-J2 cells. The results also showed EGF could reverse LPS injured ASCT2 down-expression in IPEC-J2 cells, which are similar to those for ischemic injured human intestinal epithelial cells Huang *et al*. (2007).

Conclusions

The results of the present study suggest that EGF could promot the gene and protein expression of ASCT2 in IPEC-J2 cells induced by LPS. It can be speculated that EGF promot the absorption of Gln in injured intestinal epithelial cells by activating ASCT2 expression.

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statement of conflict of interest

The authors have declared no conflict of interest.

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