Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org

Kafkas Univ Vet Fak Derg
27 (6): 803-809, 2021
DOI: 10.9775/kvfd.2021.26373

RESEARCH ARTICLE

Bioinformatic Analysis of Differentially Expressed Genes in Porcine Intestinal Epithelial Cells Infected with Transmissible Gastroenteritis Virus

Xu-xuan ZHANG [1,a] Guo-wei LAN [2,b] Zhe WANG [1,c] Chen-hao JIANG [1,d]
Jing XI [3,e] Yao LI [1,f] Jun QIAO [3,g (*)] Yan REN [1,h (*)]

[1] First Affiliated Hospital of School of Medicine, Shihezi University, Shihezi 832000, CHINA
[2] School of Chemistry and Chemical Engineering, Shihezi University, Xinjiang, 832003, CHINA
[3] School of Animal Science and Technology, Shihezi University, Shihezi 832000, CHINA
ORCIDs: [a] 0000-0003-3359-3917; [b] 0000-0002-2956-0090; [c] 0000-0003-3704-4966; [d] 0000-0002-8033-1066; [e] 0000-0002-6361-8882
[f] 0000-0002-3819-0083; [g] 0000-0001-5210-4379; [h] 0000-0002-4034-9376

Article ID: KVFD-2021-26373 Received: 09.08.2021 Accepted: 11.11.2021 Published Online: 15.11.2021

Abstract

Transmissible Gastroenteritis Virus (TGEV) infection is one of the leading causes of diarrhea in piglets. Intestinal immune system plays an important role in maintaining the intestinal mucosa's integrity and resisting infection by pathogens. However, the relationship between the immune response induced by TGEV infection and disease progression is unclear. Using the microarray data set GSE41756 from the Gene Expression Omnibus database, we analyzed porcine small intestinal epithelial cells at 6 and 12 h of TGEV infection. Differentially expressed genes (DEGs) were detected using the 'limma' R package. Gene Ontology, Kyoto Encyclopedia of Genes and Genomes pathway enrichment, and the protein-protein interaction network analyses were performed with the detected DEGs. We found 56 DEGs (47 up-regulated and 9 down-regulated) after the intersection. Pathway enrichment analysis revealed that the DEGs were mainly associated with immune response, extracellular space, cytokine activity, and positive regulation of nuclear factor-κB import into the nucleus. This revealed the strong relationships among DEGs in the tumor necrosis factor signaling pathway, inflammatory bowel disease, and influenza A. Interleukin 6 *(IL6), IL8, IL18,* tumor necrosis factor, and toll-like receptor hub genes may play important roles during TGEV infection.

Keywords: *DEGs, Immune response, TGEV, TNF, TLR2*

Bulaşıcı Gastroenterit Virüsü İle Enfekte Domuz Bağırsak Epitel Hücrelerinde Diferansiyel Gen Ekspresyonlarının Biyoinformatik Analizi

Öz

Bulaşıcı Gastroenterit Virüs (TGEV) enfeksiyonu, domuz yavrularında ishalin önde gelen nedenlerinden birisidir. Bağırsaklardaki bağışıklık sistemi, mukozal bütünlüğün korunması ve patojenlerin neden olduğu enfeksiyonlara karşı koymada önemli bir rol oynar. Ancak, TGEV enfeksiyonunun neden olduğu bağışıklık yanıt ile hastalığın ilerlemesi arasındaki ilişki açık değildir. Gene Expression Omnibus veri tabanından GSE41756 mikroarray veri setini kullanarak, 6 ve 12 saatlik TGEV enfeksiyonunda domuz ince bağırsak epitel hücrelerini analiz ettik. Diferansiyel gen ekspresyonları (DEGs), 'limma' R paketi kullanılarak tespit edildi. Tespit edilen DEG'lerin, Gen Ontology, Kyoto Genler ve Genomlar Ansiklopedisi yolak zenginleştirme ve protein-protein etkileşim ağı ile analizleri gerçekleştirildi. Kesişim noktasından sonra 56 DEG (ekspresyonu artmış 47 gen ve ekspresyonu azalmış 9 gen) saptadık. Yolak zenginleştirme analizi, DEG'lerin esasen immun yanıt, ekstraselüler boşluk, sitokin aktivitesi ve çekirdeğe nükleer faktör-κB girişinin pozitif yönde düzenlenmesi ile ilişkili olduğunu ortaya koydu. Bu, tümör nekrozis faktör sinyal yolu, inflamatuar bağırsak hastalığı ve influenza A'daki DEG'ler arasındaki güçlü ilişkileri ortaya çıkardı. İnterlökin 6 *(IL6), IL8, IL18,* tümör nekrozis faktör ve toll-like reseptör asıl genleri, TGEV enfeksiyonu sırasında önemli roller oynayabilir.

Anahtar sözcükler: *DEGs, İmmün yanıt, TGEV, TNF, TLR2e*

INTRODUCTION

Transmissible Gastroenteritis Virus (TGEV) is a contagious porcine enteropathogenic virus belonging to the alpha-coronavirus family. TGEV infects intestinal epithelial cells, resulting in severe and frequently fatal diarrhea with mortality rates reaching 100% in piglets less than 2 weeks old [1]. However, piglets that live for more than six to eight days after infection may recover, although they may have stunted growth [2] and can spread TGEV to uninfected swine

How to cite this article?

Zhang XX, Lan GW, Wang Z, Jiang CH, Xi J, Li Y, Qiao J, Ren Y: Bioinformatic analysis of differentially expressed genes in porcine intestinal epithelial cells infected with transmissible gastroenteritis virus. *Kafkas Univ Vet Fak Derg,* 27 (6): 803-809, 2021.
DOI: 10.9775/kvfd.2021.26373

(*) Corresponding Author

Tel: +9 15809935356 (J. Qiao) +9 15309914118 (R. Yan)
E-mail: qj710625@shzu.edu.cn (J. Qiao) rycb1225@163.com (R. Yan)

for several weeks. The intestinal tract is a site of digestion and nutrient absorption and acts as a barrier for harmful pathogens and toxins [3]. TGEV infection damages the small intestines and promotes secondary infection by other pathogens; thus, damaging immune function and increasing the pathogenic bacterial load. The intestinal immune system plays an essential role in maintaining intestinal mucosal homeostasis and protecting against pathogen invasion. Chronic inflammation is the cause of many diseases [4].

Genome-wide molecular profiling reveals molecular changes in the adsorption and invasion of viral infection and is an efficient approach for identifying essential genes. We performed a genome-wide molecular profiling analysis to identify essential genes and pathways associated with TGEV infection by integrating a bioinformatic analysis based on the gene expression omnibus (GEO) datasets. The data obtained indicate that some genes may continue to participate in TGEV infection.

Material and Methods

Data Sources

The GSE41756 gene expression data [5] were obtained from the Gene Expression Omnibus database (GEO; *http://www.ncbi.nlm.nih.gov/geo)*. Expression profiling arrays were generated using the GPL3533 platform. In addition, 9 porcine cells in the database, including 3 uninfected cells, 3 cells at 6 h of infection, and 3 cells at 12 h of infection were collected for further analysis.

DEG Identification

The transcripts per million approach was used for background correction and normalization. DEGs between infected and uninfected cells were processed using packages in R Software. The screening criteria for DEGs were adjusted P-value <0.05 and log2 fold change (log2FC).

GO and KEGG Pathway Enrichment Analysis of DEGs

Gene Ontology (GO) was used to annotate genes from various ontologies. The Kyoto Encyclopedia of Genes and Genomes (KEGG) *(http://www.kegg.jp/* or *http://www.genome.jp/kegg/)* data resource was used for genes and genomes with assigned corresponding functional significances. The database for annotation visualization and integrated discovery (DAVID) *(https://david.ncifcrf.gov)* was applied for GO annotation and KEGG pathway analysis with a statistically significant P-value <0.05.

PPI Network Analyses

The protein-protein interaction (PPI) network identified functional links between proteins, using a search tool to retrieve interacting genes/proteins software (version 11.0, *http://www.string-db.org)*. A composite score of >0.4 was considered a statistically significant interaction.

Module Analysis and Selection of Hub Genes

The PPI network analysis results were loaded into Cytoscape (version 3.6.1) [6] software for visual adjustment. The hub genes were identified using the CytoHubba plug-in of the Cytoscape software. CytoHubba ranks nodes based on their qualities in the network. In this study, the top five genes ranked by the MCC (Mathew correlation coefficient) method were defined as hub genes.

Results

Identification of DEGs

We analyzed DEGs at 6 and 12 h after TGEV infection. The results showed 52 up-regulated and 12 down-regulated genes at 6 h (adj P<0.05, | log (FC) | >1) *(Fig. 1)* and 75 up-regulated and 0 down-regulated genes at 12 h (adj P<0.05, | log (FC) | >1) *(Fig. 2)*. We found 47 up-regulated and 9 down-regulated genes (adj P<0.05, | log (FC) | >1) *(Table 1*, *Fig. 3)* by taking the intersections. The cluster heat-map plot and volcano plot of the DEGs are shown in *Fig. 1* and *Fig. 2*, respectively.

GO and KEGG Pathway Enrichment Analysis of DEGs

GO analysis consists of biological processes (BP), cellular components (CC), and molecular function (MF) terms. The main DEG biological process changes were collected in the immune response, extracellular space, cytokine activity, and positive regulation of nuclear factor-κB (NF-κB) were imported into the nucleus. Cell component changes were predominantly observed in the plasma membrane's extracellular region and external side (P<0.05, *Table 2)*. The KEGG pathway analysis results revealed the potent relationships among DEGs in the tumor necrosis factor (TNF) signaling pathway with inflammatory bowel disease (IBD) and influenza A (P<0.05, *Table 3)*.

PPI Network Formation

The PPI network analysis aimed to provide an understanding of the biological properties of DEG. The network comprises 49 nodes and 221 edges *(Fig. 4)*. Next, Cytoscape was used to identify the densely connected regions of PPIs for DEGs and the most significant modules selected, including the interleukin 6 *(IL6),* interleukin 8 *(IL8),* interleukin 18 *(IL18),* TNF, and toll-like receptors (TLR2) as shown in *Fig. 5*.

Discussion

This study performed gene expression profiles integration analysis from cells with or without TGEV infection to identify the DEGs, related key signaling pathways, and hub genes. Data obtained at 6 and 12 h of infection were included in the analysis. Furthermore, 56 DEGs, comprising 47 up-regulated and 9 down-regulated genes, were identified

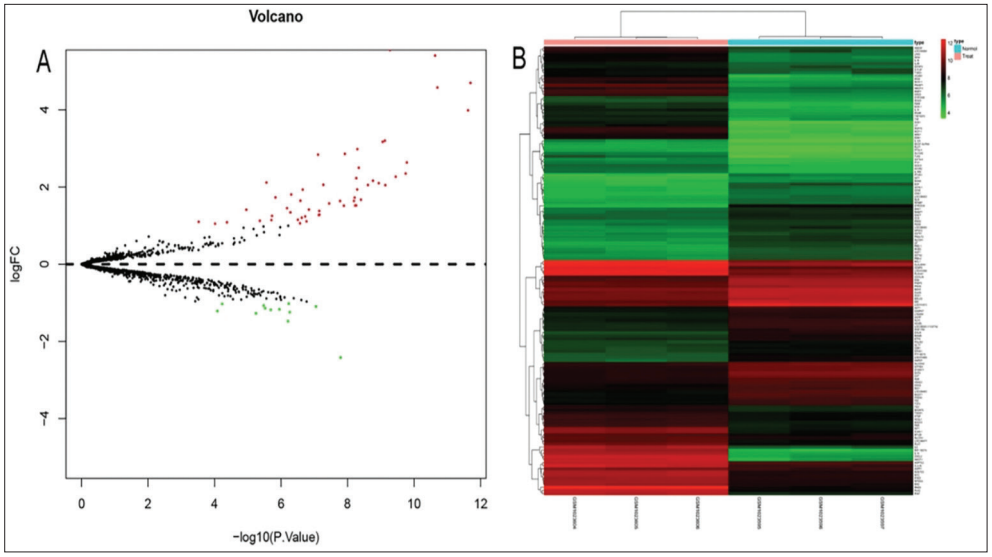

Fig 1. A. Gene expression data are presented using volcano plots. Cells were infected with TGEV for 6 h. Red points represent the up-regulated genes (n=52); green points, the down-regulated genes (n=12); and gray points, non-differentially expressed genes, **B.** Heat-map results of DEGs at 6 h

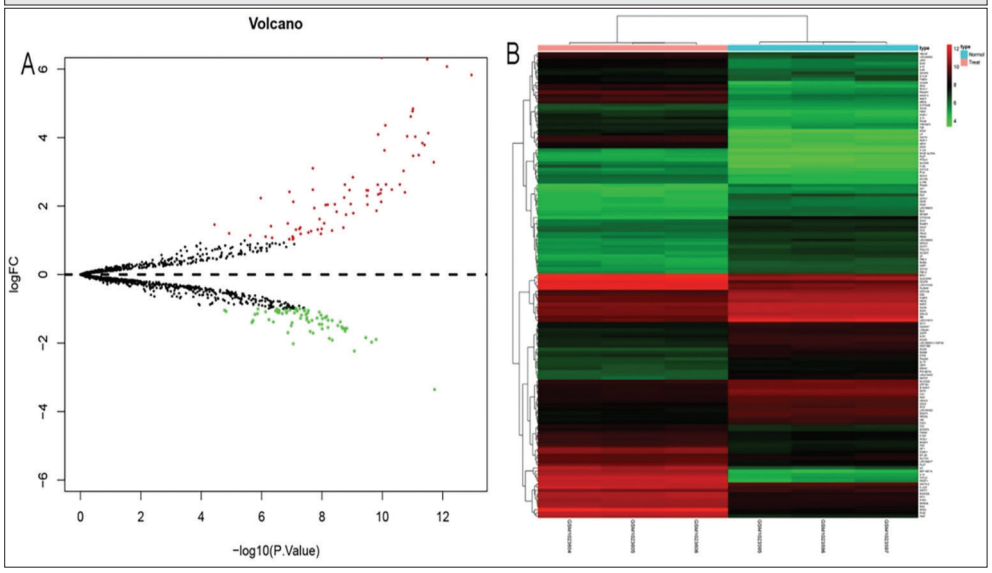

Fig 2. A. Gene expression data are presented using volcano plots. Cells were infected with TGEV for 12 h. Red points represent the up-regulated genes (n=75); green points, the down-regulated genes (n=70); and gray points, non-differentially expressed genes, **B.** Heat-map results of DEGs at 12 h

Table 1. *Screening upregulated and downregulated DEGs.expressed genes*

DEGs	Gene Symbol
Upregulated (47)	RHIV-1|PMAIP1|NFKBIA|TEC|EDN1| |YP3A46|MT-2B|IFRD1|RND3 LOC414396|MCL1|VCAM1|TNFRSF5|PLANH1|PLET|SLC5A |MMP7 IL6|OAS1|ARG1|TLR2|MCP-1|TNF|PLAT|AMCF-I|CXCL2|RANTES CEBPB|LOC396677|PIAP|IL1A|IRF1|MYC|MIP-1BETA|IL18|ACSL1 AMCF-II|IL1RN|C-JUN|PGAR|FBN1|LIF|IRG6|PLK |IL15|LOC448984|ICAM-1
Downregulated (9)	PTHR |CNN1|SLC5A1|LOC396603|UCHL1|EGF|LOC396850|UF|CYP2C33

from the GSE95368 database. The GO enrichment analysis showed that these DEGs associated with TGEV infection were mainly enriched in the immune response, extracellular space, cytokine activity, and positive regulation of NF-κB import into the nucleus. The KEGG pathway enrichment analysis found that the DEGs were mainly concentrated in the TNF signaling pathway and cytokine-cytokine receptor interaction. The *IL6*, *IL18*, *IL8*, *TLR2*, and *TNF* genes were considered hub genes for TGEV infection by the construction and module analysis of the PPI network.

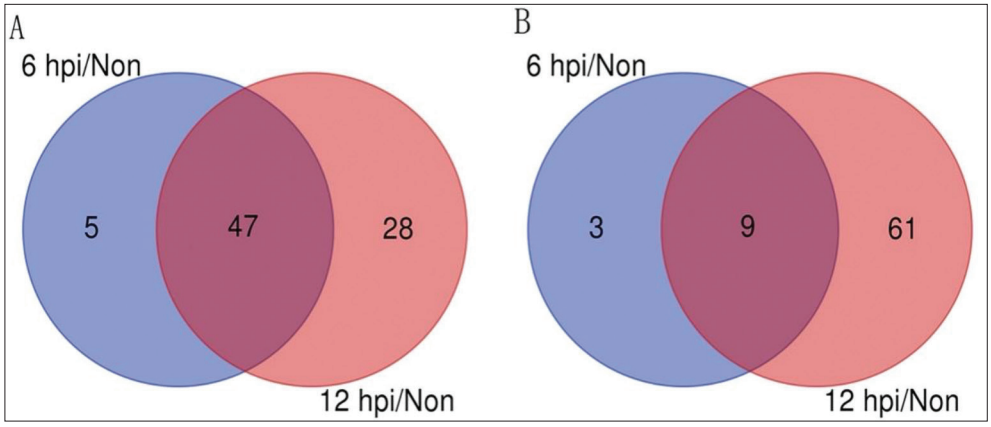

Fig 3. Differentially expressed genes

Table 2. *Go enrichment analysis of differentially expressed genes*

Term	Description	Count	P Value
GO:0006955	Immune response	8	1.95E-06
GO:0005615	Extracellular space	12	6.19E-06
GO:0005125	Cytokine activity	6	2.77E-05
GO:0042346	Positive regulation of NF-kappaB import into nucleus	3	4.98E-04
GO:0031663	Lipopolysaccharide-mediated signaling pathway	3	0.00136604
GO:0032755	Positive regulation of interleukin-6 production	3	0.001864123
GO:0006006	Glucose metabolic process	3	0.002857244
GO:0042127	Regulation of cell proliferation	4	0.00573518
GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	6	0.010185653
GO:0008284	Positive regulation of cell proliferation	4	0.019487934
GO:0006954	Inflammatory response	4	0.019487934
GO:0032496	Response to lipopolysaccharide	3	0.02110949
GO:0005576	Extracellular region	5	0.028858636
GO:0009897	External side of plasma membrane	3	0.046693492

Table 3. *KEGG pathway analysis of DEGs*

Pathway	ID	Count	P Value	Genes
TNF signaling pathway	ssc04668	10	5.37E-10	LIF|VCAM1|IL6|TNF|CEBPB|EDN1| CXCL2|NFKBIA|IL15
Rheumatoid arthritis	ssc05323	7	1.66E-06	AMCF-II|IL6|TNF|IL18|TLR2|IL15| IL1A
Legionellosis	ssc05134	6	6.58E-06	IL6|TNF|IL18|CXCL2|TLR2|NFKBIA
Malaria	ssc05144	5	7.34E-05	VCAM1|IL6|TNF|IL18|TLR2
Tuberculosis	ssc05152	7	9.50E-05	IL6|TNF|CEBPB|IL18|TLR2|IL1A
Inflammatory bowel disease (IBD)	ssc05321	5	1.38E-04	IL6|TNF|IL18|TLR2|IL1A
Pertussis	ssc05133	5	2.77E-04	AMCF-II|IL6|TNF|IRF1|IL1A
African trypanosomiasis	ssc05143	4	4.29E-04	VCAM1|IL6|TNF|IL18
Influenza A	ssc05164	6	7.48E-04	IL6|TNF|IL18|NFKBIA|OAS1|IL1A
Herpes simplex infection	ssc05168	6	0.001102297	IL6|TNF|TLR2|NFKBIA|OAS1|IL15
NOD-like receptor signaling pathway	ssc04621	4	0.001261979	IL6|TNF|IL18|NFKBIA
Cytokine-cytokine receptor interaction	ssc04060	6	0.001715922	LIF|IL6|TNF|IL18|IL15|IL1A
Hepatitis C	ssc05160	5	0.002223641	TNF|IRF1| NFKBIA|OAS1|EGF
Leishmaniasis	ssc05140	4	0.002491745	TNF|TLR2|NFKBIA|IL1A
Measles	ssc05162	5	0.002559994	IL6|TLR2|NFKBIA|OAS1|IL1A

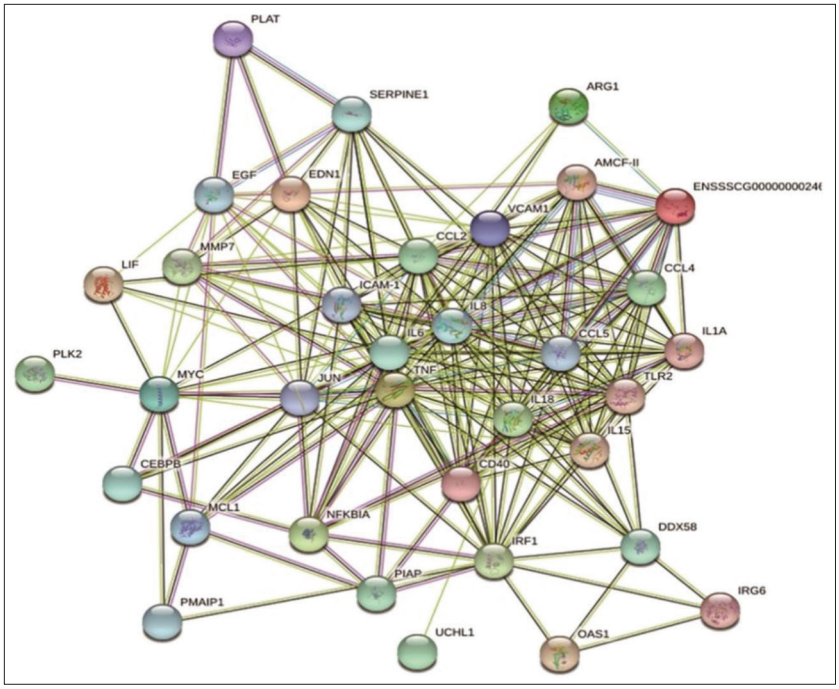

Fig 4. Results of PPI network analysis of DEGs

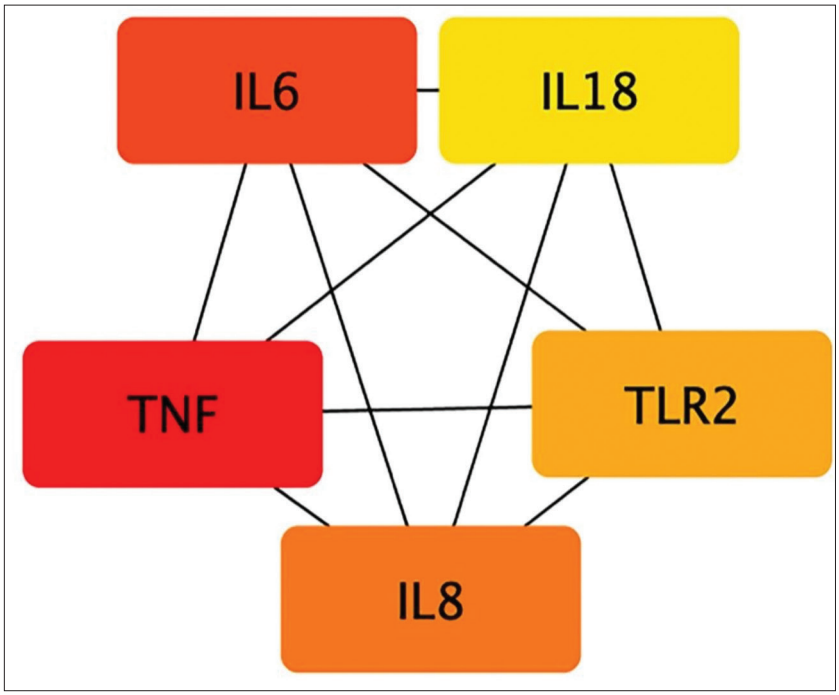

Fig 5. PPI network of the module

The invasion of viruses causes an inflammatory response, a key mediator of host responses to microbial pathogens [7]. In addition, the inflammatory factors produced promote a strong immune response. TGEV infection enhances the expression levels of *IL18, IL6, IL8,* and *TNF-α,* which are essential factors in chronic inflammation. Severe gastroenteritis is an important clinical sign of TGEV infection, and NF-κB is a key regulator of inflammation because it induces the transcription of proinflammatory genes such as *TNF-α*, *IL1β*, *IL6*, and *IL8* [8]. *IL8* is a prototypic human chemokine factor that plays an essential role in promoting cell survival and antagonizes interferon's antiviral activities [9]. Studies have shown that TGEV nucleocapsid protein up-regulates *IL8* expression in host cells by inducing endoplasmic reticulum stress and NF-κB expression [10].

Although excessive inflammation can lead to tissue damage, proinflammatory cytokines are essential for pathogen clearance. They are essential factors in chronic inflammatory responses that promote epithelial-mesenchymal transition (EMT), suggesting that persistent TGEV infection may promote EMT *in vivo* [11]. EMT is a process in which epithelial cells lose polarity and are transformed into mesenchymal cells with migration ability after cytoskeletal remodeling [12]. Inflammatory conditions induced by pathogen infections promote EMT due to the sustained activation of the NF-κB and mitogen-activated protein kinase (MAPK) modules, controlling the expression of mesenchymal markers [13]. After EMT, porcine intestinal epithelial cells were more prone to adhere to other bacteria such as enterotoxigenic *Escherichia coli* (ETEC) K88. ETEC is a common cause of enteric colibacillosis in neonatal and early-weaned pigs.

TNF-α can synergize with TGF-β and other inflammatory factors to induce EMT and control the expression of multiple other cytokines [14]. For example, TGF-β, IFN-α, and TNF-α together can induce EMT-like changes in human cancer cell lines *in vitro* [15]. In addition, in human colorectal cancer cell lines, TNF-α and TGF-β induce EMT-like changes in a NOD-like receptor family pyrin domain containing 3, Snail1 axis-dependent manner, or via an increase in expression of claudin-1 [16]. In the latter case, claudin-1 is delocalized from the membrane and activates the steroid receptor co-activator and ERK1/2 MAP kinase pathways.

The TLR family transfers extracellular antigen recognition information to intracellular antigens by recognizing their respective pathogen-related molecular patterns to initiate immune responses and induces the expression of intracellular immune-related factors [17]. TLR2 plays a role in acute and chronic infections caused by a variety of microorganisms. Studies have shown that TLR2 and TLR4 are less expressed in intestinal epithelial cells in healthy intestines [18]. However, as inflammation progresses, the expression of TLR2 and TLR4 increases, worsening the inflammation. The small intestine of pigs expresses different TLRs such as TLR1, TLR2, TLR3, TLR4, TLR6, TLR8, TLR9, and TLR10 [19]. TLR2 and TLR4 recognize viral proteins. Various TLRs exhibit different expression patterns. TLR2 is expressed abundantly in peripheral blood leukocytes and mediates host immune responses to Gram-positive bacteria and yeast via NF-κB stimulation [20]. TLR2 is expressed mainly in the lamina propria mononuclear cells and intestinal epithelial cells at low levels in normal intestines.

This study identified essential genes and related pathways by analyzing TGEV-infected porcine small intestine epithelial cells. However, the conclusions drawn in this study need to be verified in clinical trials. In addition, there is a need to research new methods on the underlying mechanism of TGEV infection from the perspective of inflammatory changes. Provide research direction and basis for further research on diarrhea caused by TGEV infection.

Availability of Data and Materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Acknowledgments

The authors wish to thank Professor Jun Qiao (School of Animal Science and Technology, Shihezi University) for his careful modification of the manuscript. The authors would also like to thank Associate Professor Yan Ren (First Affiliated Hospital of School of Medicine, Shihezi University) for his serious guidance, for providing the facilities to carry out the experiments.

Funding Information

Funding (This work was supported by grands from the National Natural Science Foundation of China (Grant No: 81760386).

Ethics Approval

Not applicable.

Competing Interests

This manuscript has not been submitted for publication elsewhere and has been approved by all co-authors. The authors declare no conflict of interest.

Authors' Contributions

ZXX analyzed the samples and statistical data and wrote the manuscript. LGW and WZ analyzed and discussed the data. JCH and XJ revised the manuscript. QJ and RY are the corresponding authors, who designed the study and supervised the entire program. All authors have read and approved the final draft of the manuscript.

References

1. Pritchard GC, Paton DJ, Wibberley G, Ibata G: Transmissible gastroenteritis and porcine epidemic diarrhoea in Britain. *Vet Rec,* 144 (22): 616-618, 1999. DOI: 10.1136/vr.144.22.616

2. Weingartl HM, Derbyshire JB: Binding of porcine transmissible gastroenteritis virus by enterocytes from newborn and weaned piglets. *Vet Microbiol,* 35 (1-2): 23-32, 1993. DOI: 10.1016/0378-1135(93)90113-I

3. Peck BCE, Shanahan MT, Singh AP, Sethupathy P: Gut microbial influences on the mammalian intestinal stem cell niche. *Stem Cells Int,* 2017:5604727, 2017. DOI: 10.1155/2017/5604727

4. Xia L, Dai L, Yu Q, Yang Q: Persistent transmissible gastroenteritis virus infection enhances enterotoxigenic *Escherichia coli* K88 adhesion by promoting epithelial-mesenchymal transition in intestinal epithelial cells. *J Virol,* 91 (21): e01256-17, 2017 DOI: 10.1128/JVI.01256-17

5. Cruz JLG, Becares M, Sola I, Oliveros JC, Enjuanes L, Zúñiga S: Alphacoronavirus protein 7 modulates host innate immune response. *J Virol,* 87 (17): 9754-9767, 2013. DOI: 10.1128/JVI.01032-13

6. Cui LJ, Bai T, Zhi LP, Liu ZH, Liu T, Xue H, Yang HH, Yang XH, Zhang M, Niu YR, Liu YF, Zhang Y: Analysis of long noncoding RNA-associated

competing endogenous RNA network in glucagon-like peptide-1 receptor agonist-mediated protection in β cells. *World J Diabetes,* 11 (9): 374-390, 2020. DOI: 10.4239/wjd.v11.i9.374

7. Qian S, Gao Z, Cao R, Yang K, Cui Y, Li S, Meng X, He Q, Li Z: Transmissible gastroenteritis virus infection up-regulates FcRn expression via nucleocapsid protein and secretion of TGF-β in porcine intestinal epithelial cells. *Front Microbiol,* 10:3085, 2020. DOI: 10.3389/fmicb.2019.03085

8. Underdahl NR, Mebus CA, Torres-Medina A: Recovery of transmissible gastroenteritis virus from chronically infected experimental pigs. *Am J Vet Res*, 36 (10): 1473-1476, 1975.

9. Jung C, Hugot JP, Barreau F: Peyer's patches: The immune sensors of the intestine. *Int J Inflam,* 2010:823710, 2010. DOI: 10.4061/2010/823710

10. Sanchez CM, Pascual-Iglesias A, Sola I, Zuñiga S, Enjuanes L: Minimum determinants of transmissible gastroenteritis virus enteric tropism are located in the n-terminus of spike protein. *Pathogens,* 9 (1):2, 2019. DOI: 10.3390/pathogens9010002

11.Bekiaris V, Persson EK, Agace WW: Intestinal dendritic cells in the regulation of mucosal immunity. *Immunol Rev,* 260 (1): 86-101, 2014. DOI: 10.1111/imr.12194

12. Hu M, Guo W, Liao Y, Xu D, Sun B, Song H, Wang T, Kuang Y, Jing B, Li K, Ling J, Yao F, Deng J: Dysregulated ENPP1 increases the malignancy of human lung cancer by inducing epithelial-mesenchymal transition phenotypes and stem cell features. *Am J Cancer Res,* 9 (1): 134-144, 2019.

13. Garidou L, Heydari S, Gossa S, McGavern DB: Therapeutic blockade of transforming growth factor beta fails to promote clearance of a persistent viral infection. *J Virol,* 86 (13): 7060-7071, 2012. DOI: 10.1128/JVI.00164-12

14. Huss DJ, Winger RC, Peng H, Yang Y, Racke MK, Lovett-Racke AE: TGF-beta enhances effector Th1 cell activation but promotes self-regulation via IL-10. *J Immunol,* 184 (10): 5628-5636, 2010. DOI: 10.4049/jimmunol.1000288

15. Pang MF, Georgoudaki AM, Lambut L, Johansson J, Tabor V, Hagikura K, Jin Y, Jansson M, Alexander JS, Nelson CM, Jakobsson L, Betsholtz C, Sund M, Karlsson MC, Fuxe J: TGF-β1-induced EMT promotes targeted migration of breast cancer cells through the lymphatic system by the activation of CCR7/CCL21-mediated chemotaxis. *Oncogene,* 35 (6): 748-760, 2016. DOI: 10.1038/onc.2015.133

16. Ricciardi M, Zanotto M, Malpeli G, Bassi G, Perbellini O, Chilosi M, Bifari F, Krampera M: Epithelial-to-mesenchymal transition (EMT) induced by inflammatory priming elicits mesenchymal stromal cell-like immune-modulatory properties in cancer cells. *Br J Cancer,* 112 (6) :1067-1075, 2015. DOI: 10.1038/bjc.2015.29

17. Park GB, Chung YH, Kim D: Induction of galectin-1 by TLR-dependent PI3K activation enhances epithelial-mesenchymal transition of metastatic ovarian cancer cells. *Oncol Rep,* 37 (5): 3137-3145, 2017. DOI: 10.3892/or.2017.5533

18. Kim J, Durai P, Jeon D, Jung ID, Lee SJ, Park YM, Kim Y: Phloretin as a potent natural TLR2/1 inhibitor suppresses TLR2-induced inflammation. *Nutrients,* 10 (7): 868, 2018. DOI: 10.3390/nu10070868

19. Pasternak JA, Aiyer VIA, Hamonic G, Beaulieu AD, Columbus DA, Wilson HL: Molecular and physiological effects on the small intestine of weaner pigs following feeding with deoxynivalenol-contaminated feed. *Toxins (Basel),* 10 (1): 40, 2018. DOI: 10.3390/toxins10010040

20. Ma SQ, Wei HL, Zhang X: TLR2 regulates allergic airway inflammation through NF-κB and MAPK signaling pathways in asthmatic mice. *Eur Rev Med Pharmacol Sci,* 22 (10): 3138-3146, 2018. DOI: 10.26355/eurrev_201805_15073