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GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

Toxoplasma gondii ROP17 inhibits the innate immune response of HEK293T cells to promote its survival

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Abstract

Toxoplasma gondii secretes a group of rhoptry-secreted kinases (ROPs), which play significant roles in promoting intracellular infection. *T. gondii* rhoptry organelle protein 17 (ROP17) is one of these important effector proteins. However, its role in modulating host cell response during infection remains poorly understood. Here, we reveal that ROP17 (genotype I) induces significant changes in the expression genes and transcription factors of host cells. HEK293T cells were transfected with PCMV-N-HA-ROP17 plasmid or empty control PCMV-N-HA plasmid. Transcriptomic analysis revealed 3138 differentially expressed genes (DEGs) in PCMV-N-HA-ROP17-transfected HEK293T cells, including 1456 upregulated, 1682 downregulated DEGs. Also, 715 of the DEGs were transcription factors (TFs), including 423 downregulated TFs and 292 upregulated TFs. Most differentially expressed TFs, whether belong to signal transduction, cancer-related pathways or immune-related pathways, were downregulated in ROP17-expressing cells. ROP17 also decreased alternative splicing events in host cells, presumably via alteration of the expression of genes involved in the alternative splicing pathway. Taken together, our findings suggest a novel strategy whereby *T. gondii* ROP17 manipulates various cellular processes, including immune response through reprogramming host gene expression to promote its own colonization and survival in the infected host cells.

Keywords ROP17 · *Toxoplasma gondii* · Differential gene expression · Transcriptome · Innate immunity

Introduction

Toxoplasma gondii is an obligate intracellular pathogen which can infect all warm-blooded vertebrates. Approximately one third of the world human population has been infected by this protozoan parasite (Hill et al. 2005; Schneider et al. 2013; Zhang et al. 2017). Although immunocompetent individuals infected with *T. gondii* show asymptomatic or mild flu-like symptoms, this parasite establishes life-long latent infection through the formation of tissue cysts in host (Weiss and Dubey

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2009). In immunocompromised patients (such as organ transplant recipients, cancer patients or AIDS patients), *T. gondii* infection can be fatal (Weiss and Dubey 2009). *T. gondii* is a highly mobile parasite that can spread among different host tissues and body fluids (Barragan and Sibley 2002; Kaye 2011). This parasite has a remarkable ability to come across placental barrier and infect fetus in utero, leading to congenital toxoplasmosis (Jones and Dubey 2010; Kaye 2011), with subsequent long-term health complications of the newly-born fetus (Moncada and Montoya 2012).

After *T. gondii* invades the host cell, *T. gondii* secretes dozens of proteins into host cell cytoplasm, including dense granule proteins (e.g., GRA1, GRA6, and GRA15) and rhoptry organelle proteins (e.g., ROP5, ROP16, ROP17, and ROP18). Most of ROPs are phosphokinases that belong to serine/threonine (S/T) kinase family (Lorenzi et al. 2016). ROPs, through protein phosphokinase activity, regulate host immune responses (Boothroyd and Dubremetz 2008; Fentress et al. 2012; Jensen et al. 2013) and other host signaling pathways (Counihan et al. 2013; Lim et al. 2012). *T. gondii* encodes a large number of proteins that belong to different ROP superfamilies. One of these superfamilies is *T. gondii* ROP2, which contains various ROP kinases, such as ROP16, ROP17, and ROP18. The roles of ROP16 and ROP18 are well-characterized (Du et al. 2014; Hunter and Sibley 2012; Jensen et al. 2013; Ong et al. 2010). ROP17 has C-terminal kinase catalytic domain (El Hajj et al. 2006; Laliberte and Carruthers 2008), and its kinase region contains a divergent RAH region that interacts with the parasitophorous vacuole membrane (PVM) of *T. gondii* (Reese and Boothroyd 2009). Previous studies showed how ROP17 protects the parasite from clearance via avoidance of IRG recruitment to the intracellular *T. gondii*-containing vacuole (Etheridge et al. 2014). Besides protecting *T. gondii* from IRG-related destruction, ROP17 of genotype I contributes to cancer immune regulation (Fox et al. 2016).

Most of previous ROP17 functional studies used gene knock-out technology to analyze the function of ROP17; however, *T. gondii* secretes a number of redundant proteins that may compensate for the missing function of ROP17. Although gene editing is useful, it may miss the opportunity to investigate the various immunoregulatory roles that ROP17 plays inside host cells. ROP17 can interact with host cytoplasmic proteins and is very likely to modify host transcription factors through its protein phosphokinase activity, which in turn can influence host gene expression. In this study, ROP17-containing eukaryotic expression plasmid was transfected into HEK293T cells and the transcriptomes of the transfected cells were determined. Our results have shown how ROP17 can manipulate host-pathogen interaction via modulating various cellular biological processes in the host cells, leading to immunomodulation and enhanced protection.

Materials and methods

Cell line and culture conditions

HEK293T cells were purchased from the ATCC and cultured in high glucose Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, USA). The medium was supplemented with 10% (*v*/*v*) fetal bovine serum (FBS; Gibco, New Zealand), 2 mM l-glutamine (HyClone), and 100 U/ml penicillin and streptomycin. Cells were grown at 37 °C in a 5% CO_2 incubator. Cells were passaged when they reach 70–80% confluency. HEK293T cell line was chosen because they allow high efficient transfection and expression of exogenous proteins.

Construction of PCMV-N-HA-ROP17 plasmid

RNA of RH strain was used as a template to amplify the coding sequence (CDS) of rhoptry protein 17 (ROP17; TOXODB: TGME49_258580), using ROP17-F (sequence: 5'-GGGGGATCCATGCTTTTACGGTCACCAACGAG CAATGAT-3′) and ROP17-R (sequence: 5'-GGGGTCGA CCTCCTTCTGTAATAAAGCCGCCTCCT-3′) primers. The PCR reaction conditions included pre-denaturation for 5 min at 98 °C; followed by 35 cycle of 98 °C for 20 s, 56 °C for 18 s, and 72 °C for 30 s; then 72 °C for 5 min; and finally, 12 °C for preservation until used for electrophoresis of PCR products. Gel Extraction kit (OMEGA, China) was used for purification of PCR target product. The purified ROP17 CDS was clone into pMD19T-simple vector (Takara, China) according to the manufacturer's instructions. The constructed pMD19T-simple-ROP17 was transformed into *E. coli* DH5α competent cells (TIANGEN, China) for amplification of pMD19T-simple-ROP17. Single colonies were randomly selected and identified using the PCR primers ROP17-F and ROP17-R. Positive clones were sequenced by Genscript Corporation (Nanjing, China). pMD19T-simple-ROP17 plasmid in *E. coli* DH5α was extracted according to the manufacturer's instructions of Endofree Plasmid Kit (TIANGEN, China) and double digested by restriction enzymes *Bam HI* and *Sa1I* at 16 °C for 24 h. The digested ROP17 fragment was then cloned into PCMV-N-HA vector and sequenced by GENEWIZ Corporation (Suzhou, China) using T3 and T7 sequencing primers. The successfully constructed PCMV-N-HA-ROP17 plasmids were extracted and purified using Endofree Maxi Plasmid Kit (TIANGEN, China).

Transfection of PCMV-N-HA-ROP17 plasmid in HEK293T cells

Xfect™ Transfection Reagent (Takara, China) was used to transfect HEK293T cells, which were maintained in T-25 cell culture flask (NEST biotechnology, China). When the cells

became 70–80% confluent, transfection was performed. Approximately 30 μg of PCMV-N-HA-ROP17 plasmid or PCMV-N-HA plasmid (empty control vector) was diluted with 300 μl Xfect™ transfection buffer, followed by mixing with 10 μl Xfect™ polymer. The mixture was vortexed for 5 s at high speed and then incubated for 10 min at ambient temperature in order to allow nanoparticle complexes to form. The nanoparticle complex solution was then added dropwise to the cell culture medium with gentle shaking. Transfected cells were incubated for 4 h, and the nanoparticle complexes were removed from the culture medium by aspiration, followed by addition of 5-ml fresh medium. After 48 h, the transfected cells were processed for indirect immunofluorescence assay and transcriptome sequencing analysis.

Indirect immunofluorescence assay

The transfected HEK293T cells were washed three times with 1× phosphate-buffered saline (PBS) and fixed with 4% fixative solution (Solarbio, China) for 10 min. The fixed cells were washed three times with PBS to remove the fixative solution and permeated with 0.1% TritonX-100 (Beyotime, China) for 20 min. The permeated cells were blocked with 5% bovine serum albumin for 1 h and then washed with PBS. Primary mouse anti-HA tag antibody (Abcam, UK) was added to cells (1:1000 dilution) and incubated overnight at 4 °C, followed by washing three times with PBS. Secondary Goat anti-mouse IgG H&L, Alexa Fluor®555 antibody (1:1000 dilution) (Abcam, UK) was added, and the cells were incubated at 37 °C for 2 h. HEK293T cell nucleus was stained with 100 ng/ml DAPI for 10 min. The cells were washed with PBS three times, and mounting medium (Solarbio, China) was added. Fluorescence microscope Axiovert 100TV (Zeiss) was used to observe the transient expression of ROP17 inside HEK293T cells, 48 h after transfection.

Global RNA-seq analysis and differentially expressed genes

The transfected HEK293T cells were submitted to BGI-Shenzhen Corporation (Shenzhen, China) for transcriptome sequencing. Total RNA was extracted with TRIzol reagent. RNA isolated from samples was obtained from three independent replicates of transfected samples. RNA concentration and quality were assessed using Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, Calif.). Poly-T oligo-attached magnetic beads were used to isolate mRNA from total RNA. Following mRNA purification, the mRNA was fragmented into small pieces using divalent cations under elevated temperature and then reverse transcribed into cDNA. Subsequent ligation of the adapter, PCR amplification, library construction, and sequencing was performed using the BGISEQ-500 platform. SOAPnuke software was used for filtering of low-quality reads, and HISAT2 software was used for mapping the reads to the reference genome. StringTie software was used to reconstruct transcripts and detect alternative splicing events. Novel gene was identified with StringTie and CPC software, which was used to predict coding potential of novel transcripts. Animal transcription factor database (AnimalTFDB) was searched for identification of transcription factors (TFs), and targets of TFs were analyzed with Trrust database. DEGseq software was used to calculate the *P* value and false discovery rate (FDR) of the differentially expressed genes (DEGs) between PCMV-N-HA-ROP17-transfected cells and PCMV-N-HA-transfected control cells. Genes with FDR ≤ 0.05 and fold change ≥ 2 were considered as DEGs. Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used for functional annotation of the DEGs. All raw data of RNA-seq data obtained in this research have been deposited in NCBI database under the access number PRJNA488318.

Validation of transcriptomics data by qRT-PCR

Gene expression data obtained by transcriptomic analysis were further verified by quantitative real-time PCR (qRT-PCR). All RNA templates used for transcriptome library construction were also reverse transcribed to cDNA using PrimerScriptTMRT reagent kit with gDNA Eraser (Takara, China). *GAPDH* was chosen as endogenous reference gene, and 24 DEGs were selected for validation. The qRT-PCR reactions were tested by the BIO-CFX96 system (Bio-Rad, USA) using abm®EvaGreen qPCR MasterMix-no dye (Applied Biological Materials Inc., Canada) according to the manufacturer's instruction. The selected genes were examined in triplicate, and the information of primers used in this study are shown in Table S1. The cycling conditions were as follows: 95 °C for 10 min, followed by 40 cycles of 94 °C for 15 s, and 60 °C for 1 min. The $2^{-\Delta\Delta CT}$ relative expression calculating method was used for calculation of the relative gene expression (Livak and Schmittgen 2001).

Western blot validation of protein expression

To examine whether ROP17 was correctly expressed in HEK293T cells, western blot was performed. The transfected cells were harvested and total proteins were extracted using ProteinExt™ Mammalian Total Protein Extraction Kit (TRAN, China). Protein concentration was quantified using the BCA Protein Assay (Pierce). Twenty-microgram total proteins and 10-μl Protein Ladder (Thermo Scientific, USA) were loaded on 12% Expressplus™ PAGE Gels (GenScript, China), and electrophoresis was performed at 110 V for 2 h. The proteins in gel were transferred to PVDF membrane

(Thermo, Germany) and blocked with 5% fat-free milk for 1 h, incubated with primary anti-HA tag antibody (Abcam, UK) overnight at 4 °C. The PVDF membrane was washed three times with 1× TBS (Solarbio, China) and incubated with Goat Anti-Mouse IgG H&L (HRP) (Abcam, UK) for 1 h at 37 °C. Following three times of 1× TBS washing, ECL reagent (Solarbio, China) was used to detect the chemiluminescence and the images were recorded using Gel Doc™ XR+ gel imaging system (BIO-RAD, USA).

Statistical analysis

Statistical significance between groups was determined by a two-tailed Student's *t* test using GraphPad Prism, version 5.0, software. A P value of < 0.05 was considered significant.

Results

Transfection and expression of ROP17 in HEK293T cells

The eukaryotic plasmid expressing ROP17 was sequenced using T3 and T7 sequencing primers. Sequencing results showed that genotype I *T. gondii* ROP17 was correctly cloned into the PCMV-N-HA plasmid (designated as PCMV-N-HA-ROP17). Indirect immunofluorescence analysis revealed that ROP17 was successfully expressed in HEK293T cells (Fig. 1a, b). However, no fluorescence or protein labeled with HA tag was detected in PCMV-N-HA plasmid-transfected HEK293T cells (Figs. 1c, d and S1). Western blot analysis showed that ROP17 protein in PCMV-N-HA-ROP17-transfected cells was about 70.1 KD, which is consistent with the expected ROP17 molecular weight (Fig. S1).

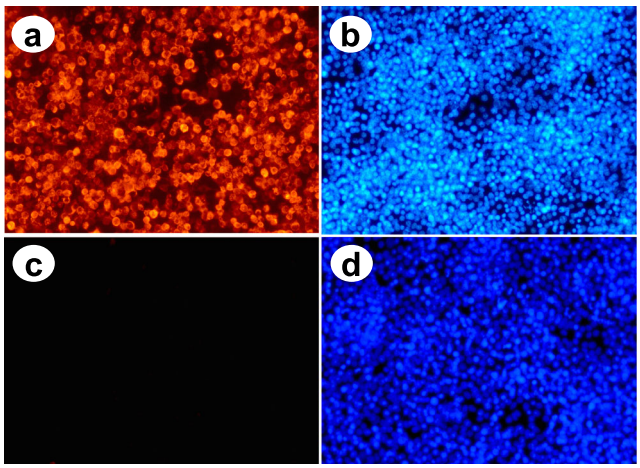

Fig. 1 Indirect immunofluorescence assay (IFA). **a** HEK293T cells transfected with PCMV-N-HA-ROP17 showed green fluorescence at × 20 magnification. **b** Nucleus of PCMV-N-HA-ROP17-transfected HEK293T cells stained with DAPI. **c** PCMV-N-HA plasmid-transfected HEK293T cells showed no green fluorescence at the same magnification. **d** PCMV-N-HA-transfected HEK293T cell nucleus stained with DAPI

RNA sequencing and identification of differentially regulated genes

Sequence analysis revealed that ~ 117 to 119 million raw reads were obtained, of these ~ 110 to 111 million reads were clean reads in each sample. About 98% reads reached Q20 standard and 92% reads reached Q30 standard. A summary of the sequencing qualities is shown in Fig. S2. Approximately 85–89% clean reads were mapped to reference human genome (Version: hg38), and 67–72% clean reads were mapped to reference human genes. More than 24,500 genes were detected in the present study, 3138 of these genes were differentially expressed in PCMV-N-HA-ROP17-transfected HEK293T cells, including 1456 upregulated and 1682 downregulated DEGs (Fig. 2a). Gene clustering showed that ROP17-transfected cells and control cells were separated into two groups (Fig. 2b). To validate our RNA-seq findings, 24 genes were examined by qRT-PCR that demonstrated the same gene expression trend, suggesting that sequencing data are robust (Fig. 2c). Alternative splicing events were also analyzed, including skipped exon (SE), alternative 5′ splicing site (A5SS), alternative 3′ splicing site (A3SS), mutually exclusive exons (MXE), and Retained Intron (RI). As shown in Table 1, the five alternative splicing events were significantly reduced in ROP17-expressing cells. Alteration of mRNA surveillance pathway that connects with alternative splicing event is shown in Fig. S3.

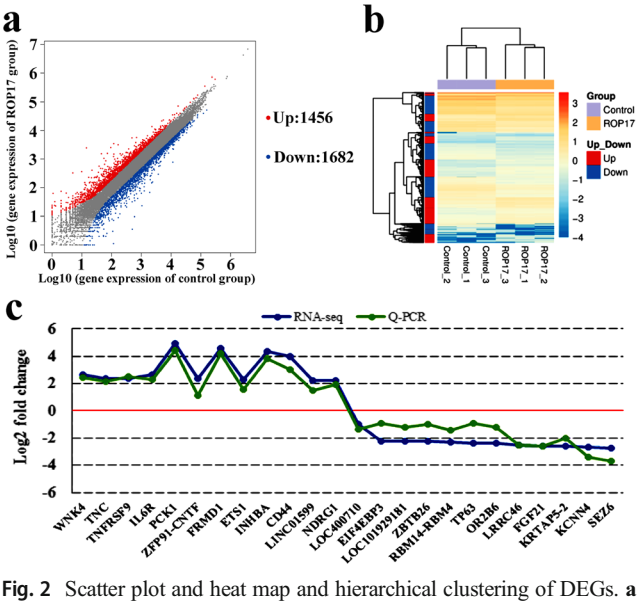

Fig. 2 Scatter plot and heat map and hierarchical clustering of DEGs. **a** Scatter plot of gene expression. **b** Heat map showing two-way hierarchical clustering of upregulated and downregulated DEGs. **c** qPCR validation of the RNA-seq results

Table 1 Alternative splicing events in control samples and ROP17-transfected samples

Sample	SE	MXE	A5SS	A3SS	RI
Control_1	56,027	12,900	5283	5419	5992
Control_2	55,326	12,675	5215	5401	5954
Control_3	50,519	11,138	5167	5285	5985
ROP17_1	38,964	7186	4699	4913	5925
ROP17_2	36,917	6589	4594	4883	5864
ROP17_3	34,366	5940	4420	4735	5806
t test *P* value	0.0014	0.001013	0.001788	0.001624	0.036695

t test *P* value was calculated with GraphPad Prism, version 5.0

SE skipped exon, *A5SS* alternative 5′ splicing site, *A3SS* alternative 3′ splicing site, *MXE* mutually exclusive exons, *RI* Retained Intron

Pathway enrichment analysis of DEGs

A total of 299 pathways were found enriched. According to the functions of pathways, these enriched pathways are distributed at six functional levels (including cellular processes, genetic information processing, environmental information processing, human diseases, metabolism, and organismal systems) and 44 subfunctional levels (Fig. 3). Signal transduction, cancer, and infection disease subfunctional levels were the top subfunctional levels that had most DEGs. One hundred fifty-two pathways included DEGs, which showed ratio (upregulated gene number/downregulated gene number) >2 or <0.5. According to DEG number, the top 10 differentially expressed pathways are shown in Fig. 4, including viral carcinogenesis, neuroactive ligand-receptor interaction, herpes simplex infection, protein digestion and absorption, basal transcription factors, nucleotide excision repair, pyrimidine metabolism, alcoholism, legionellosis, and neurotrophin signaling pathway. Seven cancer pathways were downregulated, while microRNAs in cancer pathway were upregulated (Fig. 5). Seven immune system-related pathways (cytosolic DNA-sensing pathway, B cell receptor signaling pathway, Toll-like receptor signaling pathway, natural killer cell-mediated cytotoxicity, Fc epsilon RI signaling pathway, Fc gamma R-mediated phagocytosis, and T cell receptor signaling pathway) were downregulated in ROP17-expressing HEK293T cells (Fig. 6).

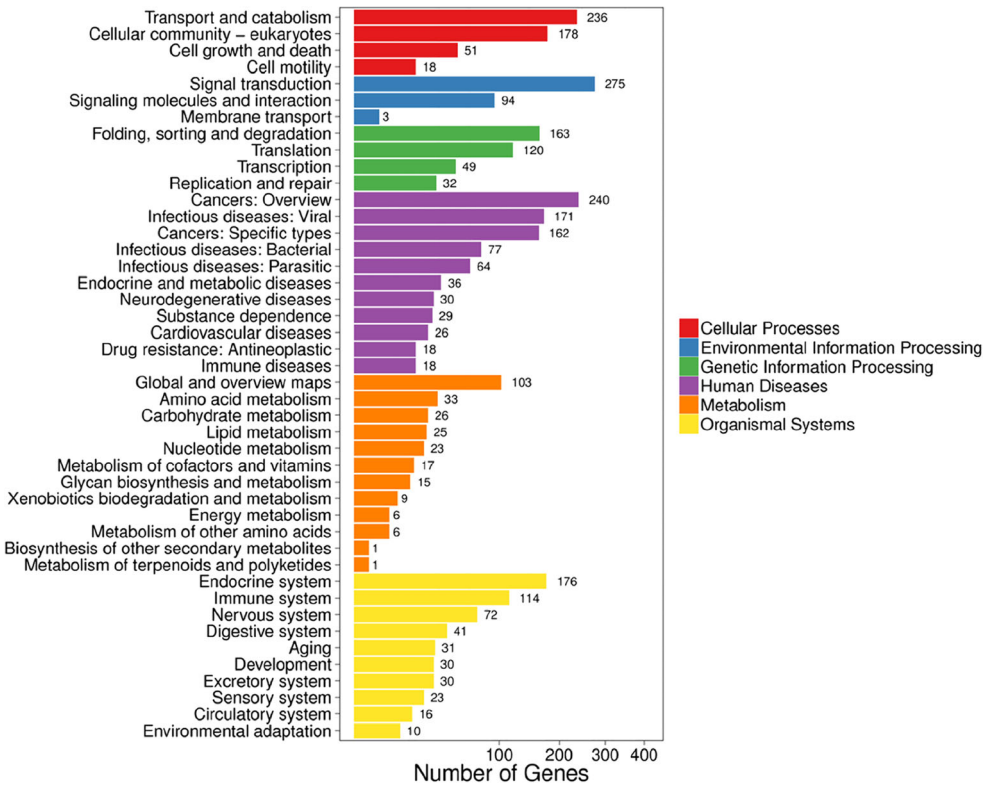

Fig. 3 Functional classifications of the enriched pathways

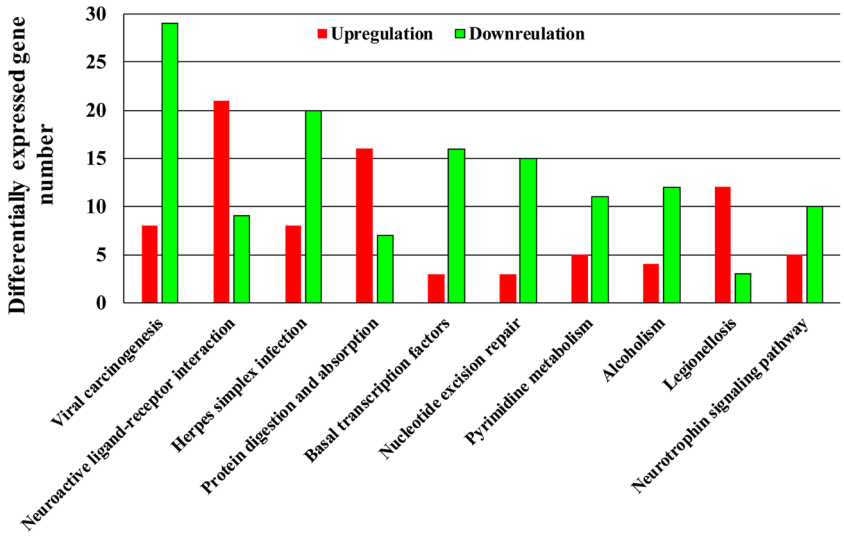

Fig. 4 Top 10 differentially expressed pathways

Differentially expressed TFs and their networks

The expressions of 715 transcription factors were significantly altered by ROP17, including 423 downregulated TFs and 292 upregulated TFs. The differentially expressed TFs were categorized into 53 TF families (Fig. 7). Most differentially expressed TFs in zf-C2H2, MBD, HMG, ARID, or ZBTB family were downregulated. zf-C2H2 family had the most

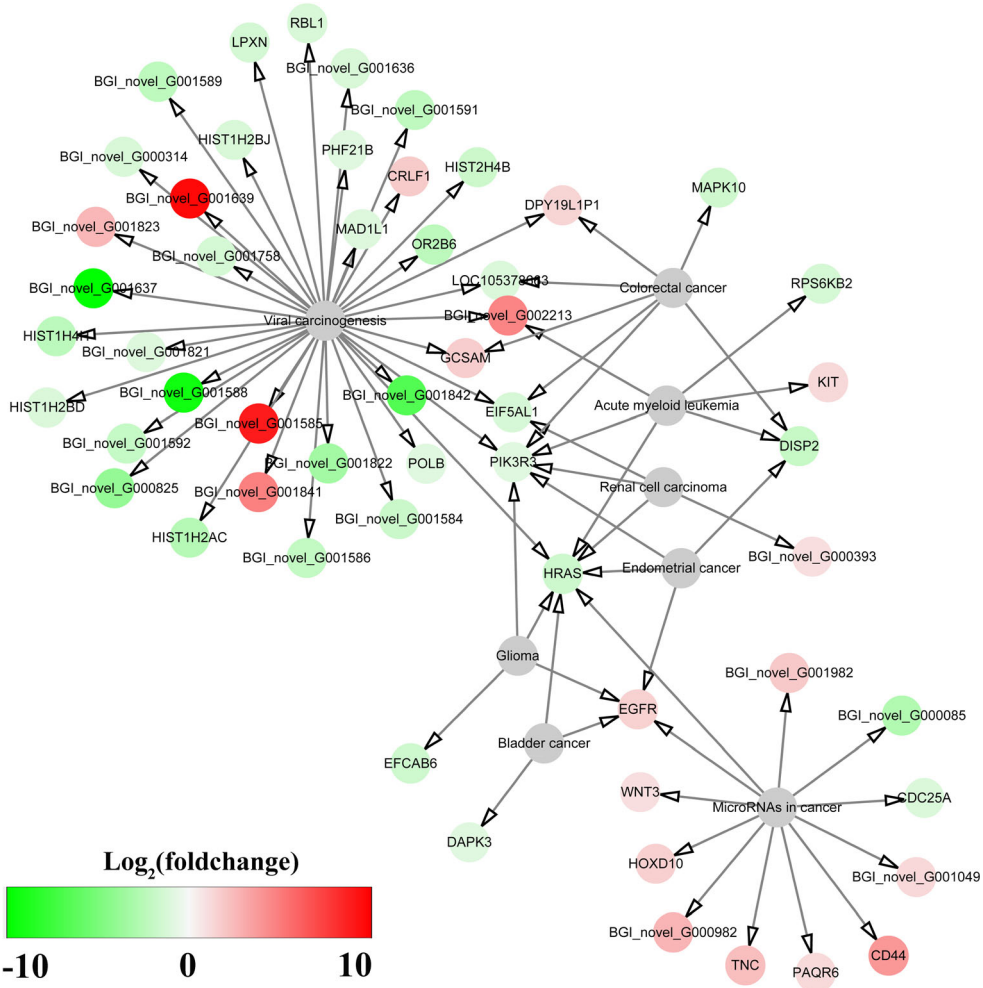

Fig. 5 Differentially expressed cancer pathways. Upregulated and downregulated genes are shown in red and green, respectively

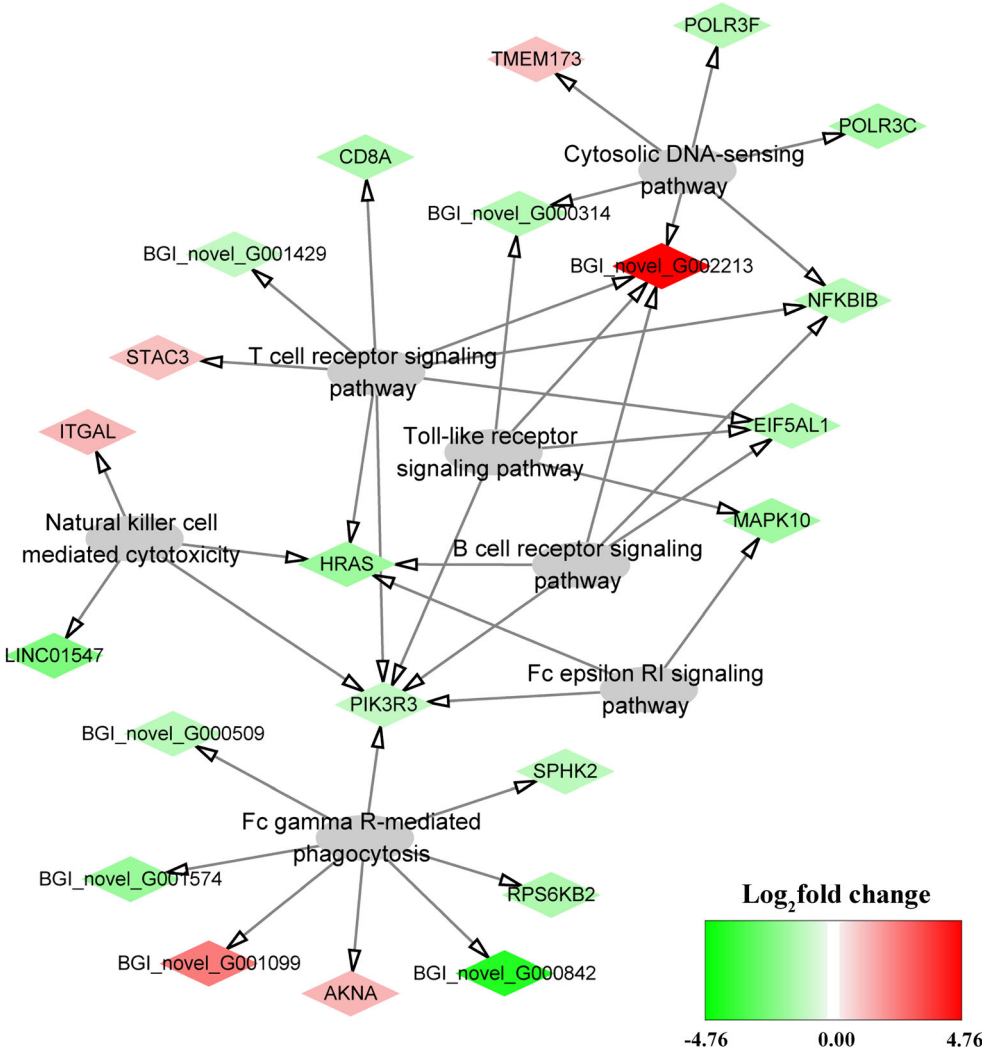

Fig. 6 Differentially expressed immune pathways. Upregulated and downregulated genes are shown in red and green, respectively

differentially expressed TFs (including 93 upregulated TFs and 197 downregulated TFs). According to Trrust database, 147 regulation pairs were found between differentially expressed TFs and DEGs (Fig. 8).

Discussion

Toxoplasma gondii has many effector proteins that play diverse and important roles in mediating the parasite interaction with the host cell. Despite evidences that *T. gondii* ROP17 mediates the protection of the parasite within the parasitophorous vacuole (PV) against the IRG pathway (Etheridge et al. 2014) and contributes to anti-cancer immunity (Fox et al. 2016), relatively little is known about how ROP17 influences global signaling pathways within host cells. The objective of the present study was to assess, comprehensively and unbiasedly, the changes in gene expression that comprise HEK293T cell response to ROP17 protein of *T. gondii* genotype I.

The immunofluorescence staining and Western blot results showed that no HA-tagged protein was expressed in control cells, whereas *T. gondii* ROP17 of genotype I was efficiently expressed in HEK293T cells (Figs. 1 and S1). RNA-seq analysis revealed that 98% of the basis had a read quality of Q20 and 92% had Q30, indicating the high quality of the obtained reads (Fig. S2). More than 60% of the reads were mapped to the reference gene, indicating that our transcriptome libraries had been successfully constructed and transcriptomic data are reliable. Alternative splicing events play important roles in biological processes (Blencowe 2006; Matlin et al. 2005). As shown in Table 1, five alternative splicing events (SE, A5SS, A3SS, MXE, and RI) were significantly reduced in ROP17-expressing cells, indicating that ROP17 may influence the regulation of host alternative splicing events, which in turns alters host biological processes. The exact mechanisms of this ROP17-perturbed host AS events remain unclear. However, it is likely that the alterations of AS events result from expressional changes of host AS relating genes, such as genes in mRNA surveillance pathway (Fig. S3).

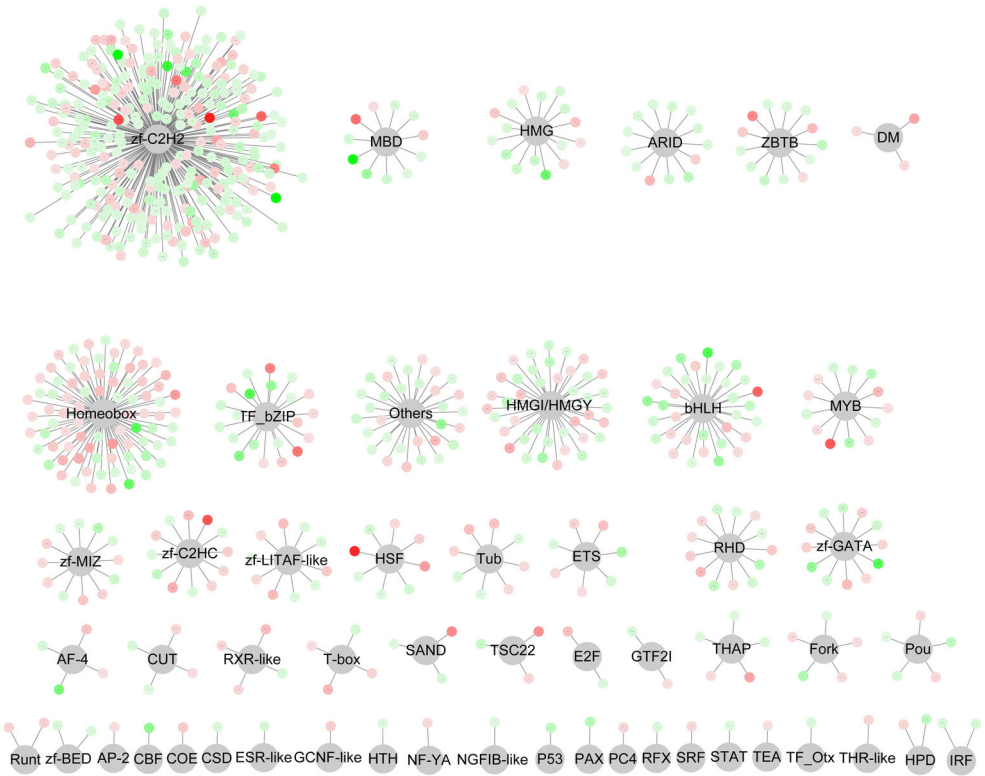

Fig. 7 Distributions of transcription factors (TFs) in each family. Upregulated and downregulated TFs are represented by red and green colors, respectively

As shown in Fig. 2, the transcriptomic profile of ROP17-expressing cells was different from control cells. Thousands of genes were differentially expressed in ROP17-expressing HEK293T cells, including 1456 upregulated and 1682 downregulated DEGs. The DEGs induced by ROP17 protein were involved in 299 pathways. Most of the DEGs participate in signal transduction, immune response, or cancer-related pathways (Fig. 3). Although the numbers of upregulated genes and downregulated genes were roughly equal, the influences of gene expression on specific pathways of host cells were different. According to the differentially expressed ratio (upregulated gene number/downregulated gene number), top 10 biological pathways that significant DEGs were involved in included viral carcinogenesis, neuroactive ligand-receptor interaction, herpes simplex infection, protein digestion and absorption, basal transcription factors, nucleotide excision repair, pyrimidine metabolism, alcoholism, legionellosis, and neurotrophin signaling pathway. Previous study showed that *T. gondii* ROP17 of genotype I contributes to anticancer immune response (Fox et al. 2016). In our study, 23 cancer-relating pathways were enriched. Six pathways, including viral carcinogenesis, colorectal cancer, acute myeloid leukemia, bladder cancer, renal cell carcinoma, endometrial cancer, and glioma, were downregulated in ROP17-expressing cells (Fig. 5). These data support the antitumor effects of ROP17 via downregulating these cancer pathways.

ROP17 contributes to the parasite immune evasion (Etheridge et al. 2014). Functional studies of ROP17 focus on its kinase activity against host immune regulators, such as IRGs or GBPs (Etheridge et al. 2014). However, the mechanisms whereby ROP17 protein contributes to *T. gondii* survival through engaging with other signaling pathways remain unknown. Our analysis did not reveal any upregulated immune pathway in ROP17-expressing cells. However, seven immune pathways were found downregulated due to the expression of *T. gondii* ROP17 protein, including cytosolic DNA-sensing pathway, B cell receptor signaling pathway, natural killer (NK) cell-mediated cytotoxicity, Fc epsilon RI signaling pathway, Fc gamma R-mediated phagocytosis, Toll-like receptor signaling pathway, and T cell receptor signaling pathway.

B cell receptor signaling pathway, Fc epsilon RI signaling pathway, and Fc gamma R-mediated phagocytosis are related to humoral immune response and participate in *T. gondii* cytolysis (Suzuki and Kobayashi 1985). NK cell-mediated cytotoxicity, T cell receptor signaling pathway, and Toll-like receptor signaling pathway play essential roles in host innate immune response against *T. gondii* infection. NK cells and T cells are major sources of IFN-γ, which stimulates immune cells to induce indoleamine 2,3-dioxygenase (IDO), inducible nitric oxide synthase (iNOS), immunity-related GTPases (IRGs), and guanylate-binding proteins (GBPs). *T. gondii* parasites are tryptophan and arginine auxotrophs. IFN-γ-mediated induction of IDO restricts *T. gondii* growth through decreasing host cellular tryptophan content. Also, iNOS generates highly toxic metabolite nitric oxide (NO) and depletes

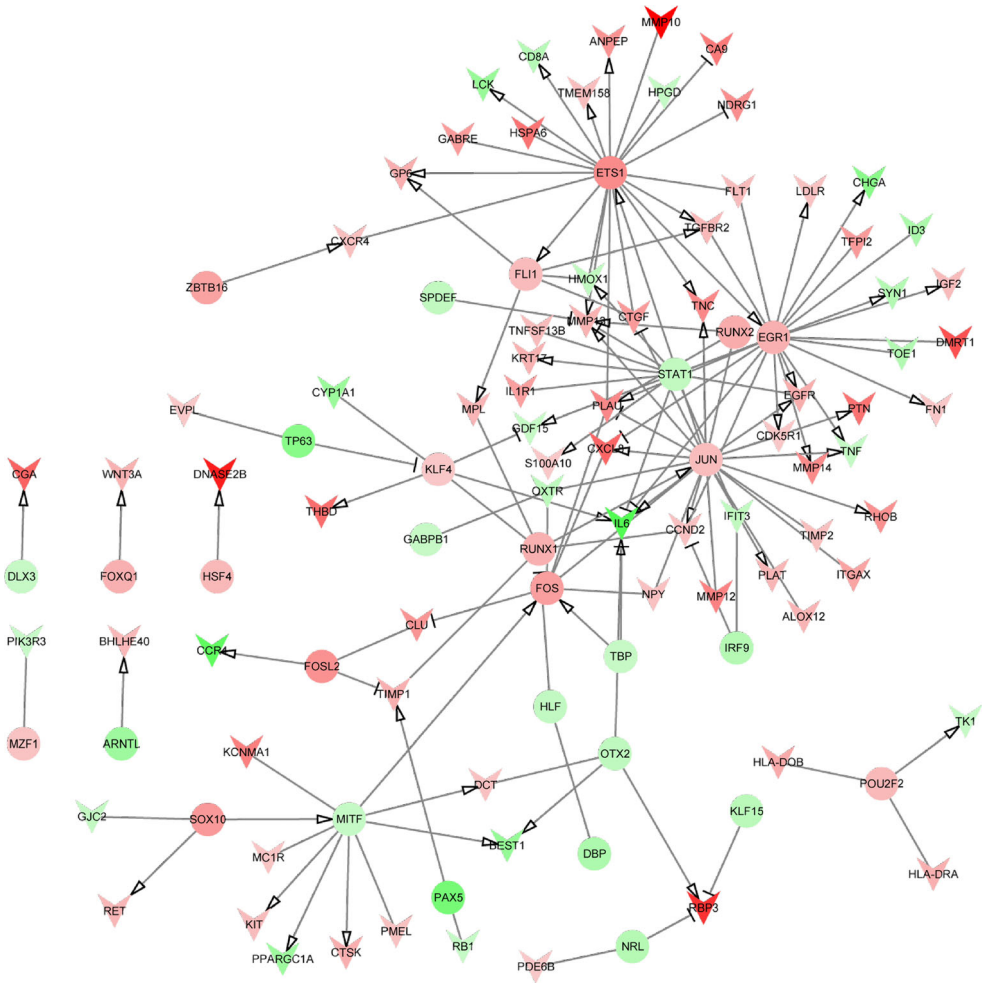

Fig. 8 Transcription factors (TFs) and their differentially expressed target genes. Differentially expressed TFs are shown in spheres, and differentially expressed target genes of TFs are shown as V shape; upregulated and downregulated genes are presented in red and green colors, respectively

host cellular cytoplasm arginine; IRGs and GBPs are recruited to and disrupt the PV of *T. gondii*. Appropriate inflammatory responses are crucial for controlling acute *T. gondii* infection. Toll-like receptor signaling pathway participates in pathogen recognition and innate immunity against *T. gondii* and activates factors to stimulate T and B cells and promote the production of IFN-γ, IL-12, and IL-1β, which are essential for parasite clearance (Yarovinsky 2014). Downregulation of these immune pathways suggests that, besides phosphorylation of the host IRGs and GBPs, ROP17 could block the expressions of components in innate and adaptive immune pathways and thus improve the likelihood of parasite colonization and establishment of infection.

It is noteworthy that the majority of the downregulated genes in immune pathways were transcription factors, such as PIK3R3, EIF5AL1, NFKBIB, and MAPK10 (Fig. 6). Following ROP17 transfection, 715 transcription factors were differentially expressed; 423 of these were downregulated. These differentially expressed TFs were categorized into 53 TF families (Fig. 7). zf-C2H2 family had the most differentially expressed TFs (290 differentially expressed TFs). Most differentially expressed TFs in zf-C2H2, MBD, HMG, ARID, and ZBTB were downregulated. According to Trrust database, we found 147 regulation pairs between differentially expressed TFs and DEGs. These findings suggest that ROP17 regulates host immune response through downregulating the expressions of key immune transcription factors, such as PIK3R3, EIF5AL1, NFKBIB, and MAPK10, and the transcription factors of zf-C2H2, MBD, HMG, ARID, or ZBTB families.

Taken together, we demonstrated that *T. gondii* virulence factor ROP17, which is known to increase the parasite resistance to clearance by IFN-γ-activated macrophages in target host cells, downregulates the activation of many immune signaling pathways and transcription factors, to inhibit immune responses during *T. gondii* infection. These findings implicate ROP17 as a negative regulator of the TLR signaling and other immune signaling pathways to dampen the host's ability to clear the parasite in vitro. Our results provide a molecular basis for the regulatory functions of ROP17 in relation to the host immune response, which could be relevant to the pathogenesis of *T. gondii* infection. A more comprehensive analysis of host responses to ROP17-expressing plasmids using proteomics and metabolomics may inform the development of novel therapies for *T. gondii* infection.

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Authors' contributions J.-J.H., X.-Q.Z., H.M.E, and H.-K.Y. conceived and designed the study, and critically revised the manuscript. J.-X.L. performed the experiment, analyzed the transcriptomic profile data and drafted the manuscript. D.C. and B.-T.Z. helped in study implementation and manuscript revision. All authors have read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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