Inflammation, Vol. 43, No. 2, April 2020 (© 2019)
DOI: 10.1007/s10753-019-01135-6

ORIGINAL ARTICLE

Integrative Bioinformatics Indentification of the Autophagic Pathway-Associated miRNA-mRNA Networks in RAW264.7 Macrophage Cells Infected with Δ*Omp25 Brucella melitensis*

Hanwei Jiao,[1,2] Yichen Luo,[1] Zhixiong Zhou,[1] Guojing Gu,[1] Bowen Li,[1] Wenjie Li,[1] Yuxuan Liu,[1] Yidan Wang,[1] Xinglong Wang,[1] Yu Zhao,[1] Li Wu,[1] Jixuan Chen,[1] Xuehong Shuai,[1] and Qingzhou Huang[1]

Abstract— Brucellosis is a zoonotic infectious disease caused by *Brucella* infection. Outer membrane protein 25 (Omp25) is closely related to the virulence and immunogenicity of *Brucella*. However, the molecular mechanism of Omp25 affecting *Brucella*-mediated macrophage autophagy remains unclear. Our previous study reported that four miRNAs (the upregulation of mmu-miR-146a-5p and mmu-miR-155-5p and downregulation of mmu-miR-149-3p and mmu-miR-5126) were confirmed and revealed the differentially expressed genes (DEGs) profile in RAW264.7 macrophage cells infected with *Brucella melitensis* Omp25 deletion mutant (Δ*Omp25 B. melitensis*). Here, we predicted the target genes of the four miRNAs by TargetScan, miRanda, and PicTar. GO and KEGG were used for functional enrichment analysis of DEGs profile to reveal the autophagic pathway-associated genes. The overlapped genes, which drawn the autophagic pathway-associated miRNA-mRNA networks by cytoscape software, were identified by intersecting with the predicted target genes and autophagic pathway-associated DEGs. qRT-PCR was performed to validate the mRNAs of networks. The results showed that the autophagic pathway-associated networks of mmu-miR-149-3p-Ptpn5, mmu-miR-149-3p-Ppp2r3c, and mmu-miR-146a-5p-Dusp16 were identified in RAW264.7 macrophage cells infected with Δ*Omp25 B. melitensis*. Our findings are of great significance in elucidating the function of Omp25, revealing the infection mechanism of *Brucella* and prophylaxising and treating brucellosis.

Hanwei Jiao, Yichen Luo and Zhixiong Zhou contributed equally to this work.

[1] Veterinary Scientific Engineering Research Center, College of Animal Science, Southwest University, Chongqing, 402460, People's Republic of China

[2] To whom correspondence should be addressed at Veterinary Scientific Engineering Research Center, College of Animal Science, Southwest University, Chongqing, 402460, People's Republic of China. E-mail: jiaohanwei@swu.edu.cn

KEY WORDS: Brucella; Omp25; RAW264.7; autophagy; miRNA-mRNA network.

INTRODUCTION

Brucellosis, is a highly contagious disease caused by the Gram-negative intracellular bacteria of the genus *Brucella*. It is one of the zoonotic diseases which could seriously endanger the safety of public sanitation around the world. *Brucella* is mainly divided into six species: *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* [1]. Oral infection is the main route of transmission of brucellosis. Infected animals can carry bacteria for a long time or even for life. Pathogenic bacteria can be discharged from milk, feces, and urine. Especially when animals abort, a large number of pathogenic bacteria will be discharged, which will pollute grasslands, livestock houses, drinking water, and feed. The susceptible animals exposed to the above pollutants can be infected through the digestive tract, respiratory tract, and damaged skin and mucosa [2–4].

Brucella outer membrane protein (Omp) can be divided into three groups according to its molecular weight: the first group is 36–38 kDa, such as Omp10 and Omp19, the second group is 31–34 kDa, such as Omp2, the third group is 25–34 kDa, such as Omp25 and Omp31 [5, 6]. The main virulence factors of *Brucella* include lipopolysaccharide (LPS) and Omp. Studies have shown that Omp25 is the virulence factor and immunogen of *Brucella*, and has a critical role in the survival and persistent infection of *Brucella*. Omp25-mediated miRNAs differentially expressed in porcine and murine macrophages, which affected *Brucella* intracellular survival by downregulating tumor necrosis factor α (TNF-α) secretion [7]. *Brucella* Omp25 promotes the BV2 microglial cells secretion of inflammatory cytokines such as interleukin-6 (IL-6) and TNF-α, and inhibits its apoptosis [8]. Type IV secretory system (T4SS) mediates intracellular viability and chronic infection of *Brucella* by regulating the expression of effector proteins, T4SS affects Omp25/Omp31 family may provide an important sight for *Brucella* to adapt rapidly to the intracellular environment [9].

Brucella Omp25 upregulated programmed death-1 (PD-1) and miR-155, miR-23b, and miR-21-5p expression and downregulated their target gene interleukin-12 (IL-12), which provided a new mechanism for monocyte/macrophage dysfunction caused by *Brucella* infection [10]. We previously reported that four miRNAs (the upregulation of mmu-miR-146a-5p and mmu-miR-155-5p and downregulation of mmu-miR-149-3p and mmu-miR-5126) were confirmed, and the differentially expressed genes (DEGs) profile was revealed in RAW264.7 macrophage cells infected with *Brucella melitensis* Omp25 deletion mutant (Δ*Omp25 B. melitensis*) [11].

Herein, we predicted the target genes of the upregulation of mmu-miR-146a-5p and mmu-miR-155-5p and downregulation of mmu-miR-149-3p and mmu-miR-5126. GO and KEGG were used for functional enrichment analysis of DEGs profile to reveal the autophagic pathway-associated genes. Venn analysis was performed to identify the autophagic pathway-associated miRNA-mRNA networks and qRT-PCR was used to validate. The autophagic pathway-associated networks of mmu-miR-149-3p-Ptpn5, mmu-miR-149-3p-Ppp2r3c, and mmu-miR-146a-5p-Dusp16 were identified in RAW264.7 macrophage cells infected with ΔOmp25 *B. melitensis*.

MATERIALS AND METHODS

Cells

RAW264.7 macrophage cell line was purchased from Shanghai cell bank of Chinese Academy of Science, and cultured with Dulbecco's Modified Eagle Medium (DMEM) (Life Technology, USA) containing 10% FBS (Gibco Company, USA), penicillin (100 U/ml) (Thermo Fisher Scientific, USA), and streptomycin (100 mg/ml) (Thermo Fisher Scientific, USA), at 5% CO_2, 37 °C.

Reconstruction of ΔOmp25 *B. melitensis* and Infection

As previous study, to reconstruct *B. melitensis* M5-90 *Omp25* gene deletion mutant (Δ*Omp25 B. melitensis*) [11], briefly, we reconstructed suicide plasmids using suicide vector pGEM-7ZF(+), the *Kana*^{r} gene fragment which replaced the coding region of *Omp25* gene of *B. melitensis* M5-90, ensured that the reading frame remains unchanged, and reconstructed *B. melitensis* M5-90 *Omp25* gene deletion (Δ*Omp25 B. melitensis*) by homologous recombination.

RAW264.7 macrophage cells respectively infected with *B. melitensis* M5-90 and Δ*Omp25 B. melitensis* for 4 h according to our previous study [11]. And the total RNAs were extracted according to the purelink™ RNA mini kit (Life Technology, USA) instructions to validate

the genes of the autophagic pathway-associated miRNA-mRNA networks.

Prediction Target Genes of the Confirmed Four miRNAs

Three online databases of TargetScan (http://www.targetscan.org/), miRanda (http://www.microrna.org/microrna/home.do), and PicTar (http://www.pictar.org/) were used to predict target genes of the confirmed mmu-miR-146a-5p, mmu-miR-155-5p, mmu-miR-149-3p, and mmu-miR-5126. Potential target genes for each miRNAs were the intersection of three online database predictions as previously described [11].

GO and KEGG Functional Enrichment of the Differentially Expressed Genes (DEGs) Profile

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to functionally enrich the differentially expressed genes (DEGs) profile to identify the autophagic pathway-associated DEGs. GO is an internationally standardized classification system of gene functions. GO has three ontologies, which describe the molecular function of genes, cellular components, and biological processes. GO enrichment plot can visually show the percentage of target genes annotated to the same GO. GO enrichment scatter plot indicated for autophagy-related genes in DEGs. KEGG is the main public database for pathway, KEGG enrichment analysis for DEGs to further reveal the autophagic pathway-associated genes.

Venn Analysis and the Autophagic Pathway Associated miRNA-mRNA Networks Identification

The results of predicted target genes of the confirmed mmu-miR-146a-5p, mmu-miR-155-5p, mmu-miR-149-3p, and mmu-miR-5126 by three online databases, intersected the autophagic pathway-associated DEGs by GO and KEGG functional enrichment analysis to obtain the overlapped genes, which identified the autophagic pathway-associated miRNA and its potential target mRNA. miRNA-mRNA networks were drawn by cytoscape online software (http://www.cytoscape.org/).

qRT-PCR Validation for the Genes of the Autophagic Pathway-Associated miRNA-mRNA Networks

The high quality total RNAs of RAW264.7 macrophage cells-infected *B. melitensis* M5-90 and Δ*Omp25 B. melitensis* were used as the template to obtain cDNA according to the M-MLV first-strand synthesis kit (Life Technology, USA) instructions; the specific primers were designed and synthesized as shown in Table 2. qRT-PCR was used to validate the mRNAs of the autophagic pathway-associated miRNA-mRNA networks; GAPDH was used for an internal control. Each sample was repeated three times.

Statistical Analysis

The *t* test or one-way ANOVA was performed for statistical analysis of data. Independent sample *t* test was used for comparison within the group. One-way ANOVA was used for comparison between different groups. $P < 0.05$ was considered statistically significant. $P < 0.01$ was considered very statistically significant.

RESULTS

The Significantly Differentially Expressed Genes (DEGs) Profile of RAW264.7 Macrophage Cells Infected with ΔOmp25 *B. melitensis*

RAW264.7 macrophage cells were infected with *B. melitensis* M5-90 and Δ*Omp25 B. melitensis*, respectively. Total RNAs were extracted to perform the agilent transcriptome sequencing analysis experiment according to our pervious study. As shown in Fig. 1a, red dots represented the upregulated DEGs and green dots represented downregulated DEGs in RAW264.7 macrophage cells affected by Δ*Omp25 B. melitensis* infection. As shown in Fig. 1b, red dots represented the DEGs with log2 (fold change) (Omp25 *vs* M5-90) > 2.0, and green dots represented the DEGs with log2 (fold change) (Omp25 *vs* M5-90) < − 2.0 in RAW264.7 macrophage cells affected by Δ*Omp25 B. melitensis* infection.

Venn Analysis Identified the Autophagic Pathway-Associated mRNAs in RAW264.7 Macrophage Cells Affected by Δ*Omp25 B. melitensis* Infection

The results of predicted target genes of the confirmed 4 miRNAs intersected the autophagic pathway-associated DEGs by GO and KEGG functional enrichment analysis. The overlapping part was the autophagic pathway-associated mRNAs in RAW264.7 macrophage cells affected by Δ*Omp25 B. melitensis* infection, As shown in Table 1 and Fig. 2, there were 6 genes, they were *Dnase2b* (deoxyribonuclease II beta), *Mras* (muscle and microspikes RAS), *Ptpn5* (protein tyrosine phosphatase, non-receptor type 5), *Dusp16* (dual specificity phosphatase 16), *Nfkb1* (nuclear

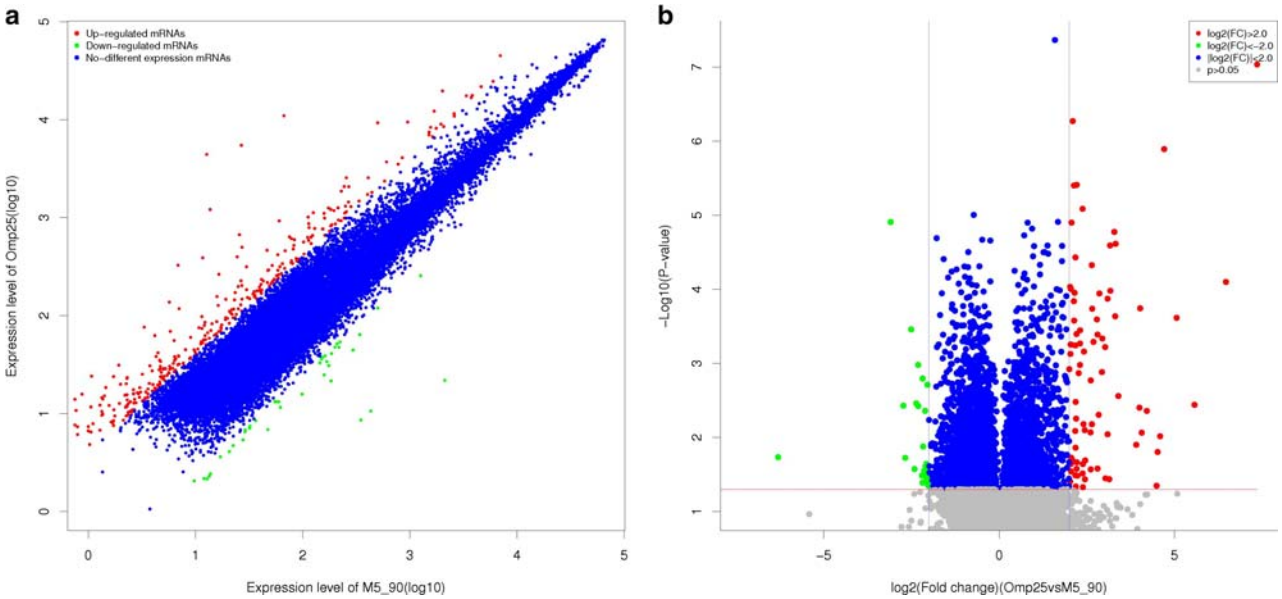

Fig. 1. The significantly differentially expressed genes (DEGs) profile of RAW264.7 macrophage cells infected with *B. melitensis* M5-90 and Δ*Omp25 B. melitensis*, respectively. **a** Scatter plot of DEGs. Red dots represented the upregulated DEGs, and green dots represented downregulated DEGs. **b** Volcano plot of DEGs. Red dots represented the DEGs with log2 (fold change) (Omp25 *vs* M5-90) > 2.0, and green dots represented the DEGs with log2 (fold change) (Omp25 *vs* M5–90) < − 2.0

factor of kappa light polypeptide gene enhancer in B cells 1, p105), and *Ppp2r3c* (protein phosphatase 2, regulatory subunit B″, gamma).

Bioinformatics Analysis of the Autophagic Pathway-Associated miRNA-mRNA Networks in RAW264.7 Macrophage Cells Infected with ΔOmp25 *B. melitensis*

Venn analysis results showed that there were six mRNAs-associated autophagic pathway, and the six mRNAs were the target genes of two miRNAs. miRNA-mRNA networks were drawn by cytoscape online software. The results of Fig. 3 indicated that there were 5 miRNA-mRNA network-associated autophagic pathways in RAW264.7 macrophage cells infected with Δ*Omp25 B. melitensis*; they were mmu-miR-149-3p-Mars, mmu-miR-149-3p-Ptpn5, mmu-miR-149-3p-Ppp2r3c, mmu-miR-146a-5p-Dnase2b, and mmu-miR-146a-5p-Dusp16.

qRT-PCR Validation and Identification the Autophagic Pathway-Associated miRNA-mRNA Networks in RAW264.7 Macrophage Cells Infected with ΔOmp25 *B. melitensis*

Specific primers were designed, as shown in Table 2, qRT-PCR was used to validate the relative expression level

Table 1. The autophagic pathway-associated mRNAs in RAW264.7 macrophage cells affected by Δ*Omp25 B. melitensis* infection by agilent transcriptome sequencing analysis

		Group1 *B. melitensis* M5-90	Group2 Δ*Omp25 B. melitensis*	
Gene symbol	*p* value	Mean	Mean	Log2 (G2/G1)
Dnase2b	2.54E-02	8.38	19.09	1.19
Mras	1.49E-02	25.39	75.84	1.58
Ptpn5	4.54E-02	725.97	1476.81	1.02
Dusp16	2.82E-03	253.67	627.60	1.31
Nfkb1	1.21E-04	241.09	669.76	1.47
Ppp2r3c	2.21E-02	980.23	1153.29	0.23

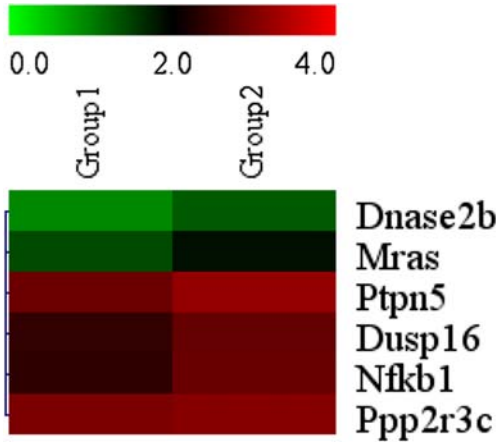

Fig. 2. Heat-map of the autophagic pathway-associated target mRNAs in RAW264.7 macrophage cells affected by Δ*Omp25 B. melitensis* infection. Group1 represented the three times repeated samples of RAW264.7 macrophage cells infected with *B. melitensis* M5-90, and Group2 represented the three times repeated samples of RAW264.7 macrophage cells infected with Δ*Omp25 B. melitensis*, respectively.

of the mRNA of the autophagic pathway-associated miRNA-mRNA networks. The confirmed mRNAs were Ptpn5, Dusp16, and Ppp2r3c, which demonstrated that the autophagic pathway-associated mmu-miR-149-3p-Ptpn5, mmu-miR-149-3p-Ppp2r3c, and mmu-miR-146a-5p-Dusp16 networks were identified in RAW264.7 macrophage cells infected with Δ*Omp25 B. melitensis*, as shown in Fig. 4.

DISCUSSION

Brucella is a Gram-negative intracellular bacteria, which can enter, survive, and proliferate in a variety of host cell types including macrophages and dendritic cells [12]. *Brucella* controls the transport of intracellular membrane, thus promoting its survival and growth in cells. When *Brucella* is absorbed by phagocytosis, it attaches to cell membrane and forms the *Brucella*-containing vacuole (BCV), which spreads along the endocytosis pathway in the first 8 h after infection and has limited fusion with lysosomes [13]. Although, the intracellular cycle of *Brucella* is replicated from the endoplasmic reticulum (ER) within 12 to 24 h postinfection [14–18]. However, the mechanism of intracellular parasitic reproduction and the process of autophagy mediated by *Brucella* are still unclear.

Omp25 is an important virulent protein and immunogen of *Brucella*. It has been reported in mice as an antigen to protect *Brucella* infection by inducing Th1 and Th2 immune responses. Studies have shown that the complex of Omp25 and other proteins, as a prospective and effective immunogen, can induce cell-mediated and humoral immune responses to brucellosis [19]. Omp25 affects the structural stability of *Brucella* and plays an important role in the activation of mitogen-activated protein kinase (MAPK) signal pathway by *Brucella* [20]. *B. melitensis* M5-90 can cause abortion of pregnant female and livestock, and its virulence is still strong. Maybe the Δ*Omp25 B. melitensi*-deleted strain will have more prospects and value for vaccine application.

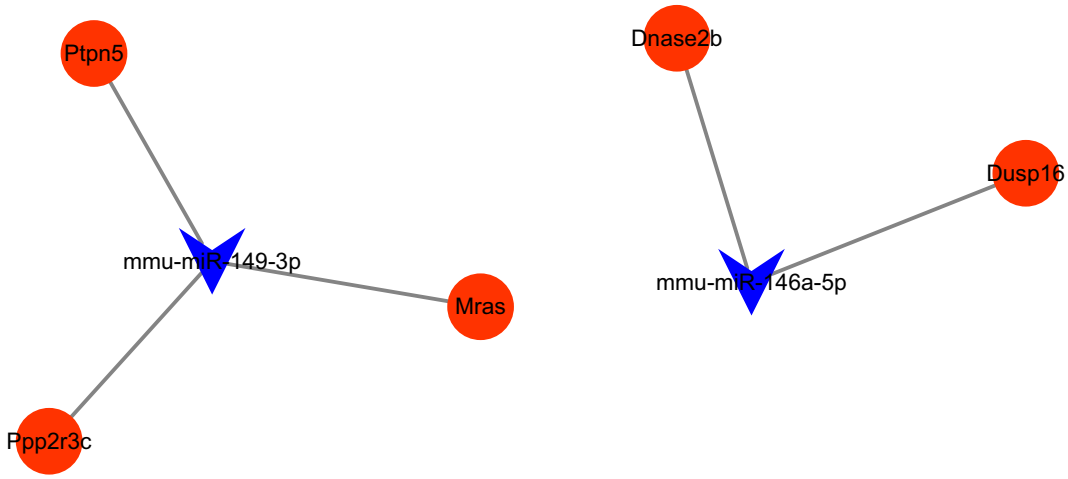

Fig. 3. The autophagic pathway-associated miRNA-mRNA networks by bioinformatics analysis in RAW264.7 macrophage cells infected with Δ*Omp25 B. melitensis*. Green arrow represented downregulated miRNAs, and the red dot represented their target genes associated with the autophagy pathway in RAW264.7 macrophage cells infected with Δ*Omp25 B. melitensis*.

Table 2. The primers designed for qRT-PCR validation for mRNAs of the autophagic pathway-associated miRNA-mRNA networks

Gene symbol	Genbank ID	Primer sequence (5′-3′)
Dnase2b	ENSMUST00000029836	F: GTTCCCAAGTTTCCCCCAGT R: TGCGGAGCCCTTTATGGAAA
Mras	NM_008624	F: CCGATGAGCAAACCCCTTCT R: CCCTTTGACCTAAGAGCGCA
Ptpn5	ENSMUST00000130381	F: TGTTGAACCATCTGGGACCTG R: AGCAAGAAAGGGTCCAGAGC
Dusp16	NM_130447	F: CCAGACTGGAATGTCAGGGC R: TGTTGGTCCCATCCAGTGTG
Nfkb1	NM_008689	F: AGTGGGAATTTCCAGCCAGG R: GCGTGCAGGTGGATGTTTTT
Ppp2r3c	NM_021529	F: TGGCTTCTGGACCTACGAGA R: TCTGACTGCAACCAACACTC
GAPDH	NM_008084	F: TGTGTCCGTCGTGGATCTGA R: CCTGCTTCACCACCTTCTTGA

There are few reports that *Brucella* Omp25 affects autophagy of macrophages or other target cells. We used bioinformatics software to analyze and predict the effect of *Brucella* Omp25 on the miRNA-mRNA regulatory networks associated with RAW264.7 macrophage cells autophagy. mmu-miR-149-3p-Ptpn5, mmu-miR-149-3p-Ppp2r3c, and mmu-miR-146a-5p-Dusp16 networks were identified. Ptpn5 plays an important role in neurodegenerative diseases and neuropsychiatric diseases, and is an attractive new target for the treatment of these diseases [21]. Ppp2r3c is a regulatory subunit of protein phosphatase; its dephosphorylation can bind to P-glycoprotein and affect the drug resistance of tumors [22]. *Mycobacterium tuberculosis* initiates the inhibition of c-Jun N-terminal kinases (JNK)-dependent autophagy by acetylating Dusp16/mitogen-activated protein kinase phosphatase-7 (MKP-7), which mediates the host immune response and improves the survival rate of mycobacterium in macrophages [23]. During the cold storm, the subcutaneous fat bank initiated miR-149-3p targeting regulation of PR domain containing 16 (PRDM16) expression to preserve energy and maintain balance [24]. mmu-miR-146a-5p plays an important regulatory role in aspergillus-induced central nervous system inflammation [25].

For the first time, we have identified three miRNA-mRNA networks associated with the autophagic pathway of macrophage cells infected with Δ*Omp25 B. melitensis*. Although bioinformatics analysis and prediction are false positive, follow-up double-luciferase reporter gene experiments should further validate these three networks. Our findings not only lay a foundation for further elucidating the molecular mechanism of *Brucella* Omp25 affecting macrophage autophagy, but also provide a new way for the prophylaxis and treatment of brucellosis.

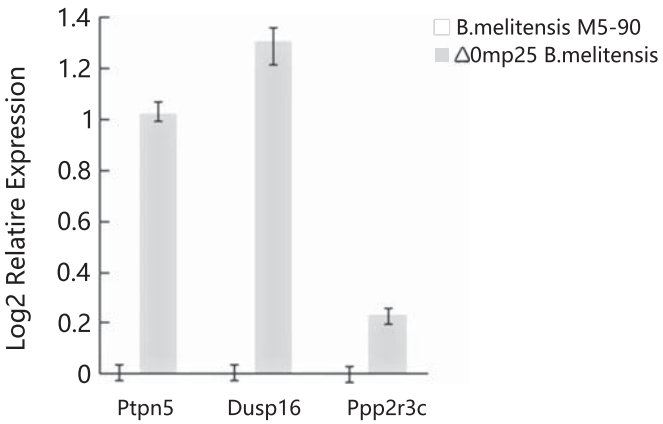

Fig. 4. qRT-PCR validated the autophagy pathway-associated target mRNAs of the miRNA-mRNA networks

FUNDING INFORMATION

This study was financially supported by the National Science Foundation for Young Scientists of China (No. 31802215), the Chongqing Research Program of Basic Research and Frontier Technology (No. cstc2018jcyjA0807), and the Fundamental Research Funds for the Central Universities (Nos. XDJK2019C024, XDJK2019D013).

REFERENCES

1. Vergnaud, G., Hauck, Y., Christiany, D., Daoud, B., Pourcel, C1., Jacques, I., Cloeckaert, A., and Zygmunt, M.S. Genotypic expansion within the population structure of classical Brucella species revealed by MLVA16 typing of 1404 Brucella isolates from different animal and geographic origins, 1974–2006. 2018. *Front Microbiol* 9: 1545.
2. Muñoz, P.M., V. Mick, L. Sacchini, A. Janowicz, M.J. de Miguel, M.A. Cherfa, C.R. Nevado, G. Girault, S. Andrés-Barranco, M. Jay, E. Di Giannatale, K. Zilli, M. Ancora, A. Dondo, S. Zoppi, M.C. Arnal, M. Tittarelli, F. De Massis, B. Garin-Bastuji, J.M. Blasco, and G. Garofolo. 2019. Phylogeography and epidemiology of Brucella suis biovar 2 in wildlife and domestic swine. *Veterinary Microbiology* 233: 68–77.
3. Zhang, N., Zhou, H., Huang, D.S., and Guan, P. Brucellosis awareness and knowledge in communities worldwide: a systematic review and meta-analysis of 79 observational studies. 2019. *PLoS Negl Trop Dis* 13: e0007366.
4. Franc, K.A., Krecek, R.C., Häsler, B.N., and Arenas-Gamboa, A.M. Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. 2018. *BMC Public Health* 18: 125.
5. Guzman-Verri, C., Manterola, L., Sola-Landa, A., Parra, A., Cloeckaert, A., Garin, J., Gorvel, J.P., Moriyon, I., Moreno, E., and Lopez-Goni, I. The two-component system BvrR/BvrS essential for Brucella abortus virulence regulates the expression of outer membrane proteins with counterparts in members of the Rhizobiaceae. 2002. Proceedings of the National Academy of Sciences of the United States of America 99: 12375–12380.
6. Cloeckaert, A., J.M. Verger, M. Grayon, and N. Vizcaíno. 1996. Molecular and immunological characterization of the major outer membrane proteins of Brucella. *FEMS Microbiology Letters* 145: 1–8.
7. Luo, X., Zhang, X., Wu, X., Yang, X., Han, C., Wang, Z., Du, Q., Zhao, X., Liu, S.L., Tong, D., and Huang, Y. Brucella downregulates tumor necrosis factor-α to promote intracellular survival via Omp25 regulation of different microRNAs in porcine and murine macrophages. 2018. *Front Immunol* 8: 2013.
8. Ma, Q.L., A.C. Liu, X.J. Ma, Y.B. Wang, Y.T. Hou, and Z.H. Wang. 2015. Brucella outer membrane protein Omp25 induces microglial cells in vitro to secrete inflammatory cytokines and inhibit apoptosis. *International Journal of Clinical and Experimental Medicine* 8: 17530–17535.
9. Wang, Y., Z. Chen, F. Qiao, Z. Zhong, J. Xu, Z. Wang, X. Du, Q. Qu, J. Yuan, L. Jia, H. Song, Y. Sun, and L. Huang. 2010. The type IV secretion system affects the expression of Omp25/Omp31 and the outer membrane properties of Brucella melitensis. *FEMS Microbiology Letters* 303: 92–100.
10. Cui, B., Liu, W., Wang, X., Chen, Y., Du, Q., Zhao, X., Zhang, H., Liu, S.L., Tong, D., and Huang, Y. Brucella Omp25 upregulates miR-155, miR-21-5p, and miR-23b to inhibit interleukin-12 production via modulation of programmed death-1 signaling in human monocyte/macrophages. 2017. *Front Immunol* 8: 708.
11. Zhu, H., Jiao, H., Nie, X., Li, B., Xu, K., Pang, F., Cao, R., Zhu, S., Yang, X., Zhang, Z., Peng, D., Li, Y., Li, G., Huang, H., Chen, C., Du, L., and Wang, F. Alterations of microRNAs and their predicted targeting mRNAs expression in RAW264.7 macrophages infected with Omp25 mutant Brucella melitensis. 2018. *Innate Immun* 24: 382–389.
12. Starr, T., R. Child, T.D. Wehrly, B. Hansen, S. Hwang, C. López-Otin, H.W. Virgin, and J. Celli. 2012. Selective subversion of autophagy complexes facilitates completion of the Brucella intracellular cycle. *Cell Host & Microbe* 11: 33–45.
13. Starr, T., T.W. Ng, T.D. Wehrly, L.A. Knodler, and J. Celli. 2008. Brucella intracellular replication requires trafficking through the late endosomal/lysosomal compartment. *Traffic* 9: 678–694.
14. Arellano-Reynoso, B., N. Lapaque, S. Salcedo, G. Briones, A.E. Ciocchini, R. Ugalde, E. Moreno, I. Moriyón, and J.P. Gorvel. 2005. Cyclic beta-1,2-glucan is a Brucella virulence factor required for intracellular survival. *Nature Immunology* 6: 618–625.
15. Bellaire, B.H., R.M. Roop, and J.A. Cardelli. 2005. Opsonized virulent Brucella abortus replicates within nonacidic, endoplasmic reticulum-negative, LAMP-1-positive phagosomes in human monocytes. *Infection and Immunity* 73: 3702–3713.
16. Celli, J., Salcedo, S.P., and Gorvel, J.P. Brucella coopts the small GTPase Sar1 for intracellular replication. 2005. Proceedings of the National Academy of Sciences of the United States of America 102: 1673–1678.
17. Salcedo, S.P., Marchesini, M.I., Lelouard, H., Fugier, E., Jolly, G., Balor, S., Muller, A., Lapaque, N., Demaria, O., Alexopoulou, L., Comerci, D.J., Ugalde, R.A., Pierre, P., and Gorvel, J.P. Brucella control of dendritic cell maturation is dependent on the TIR-containing protein Btp1. 2008. *PLoS Pathog* 4: e21.
18. Fugier, E., Salcedo, S.P., de Chastellier, C., Pophillat, M., Muller, A., Arce-Gorvel, V., Fourquet, P., and Gorvel, J.P. The glyceraldehyde-3-phosphate dehydrogenase and the small GTPase Rab 2 are crucial for Brucella replication. 2009. *PLoS Pathog* 5: e1000487.
19. Paul, S., Peddayelachagiri, B.V., Nagaraj, S., Konduru, B., and Batra, H.V. Protective and therapeutic efficacy study of divalent fusion protein rL7/L12-Omp25 against *B. abortus* 544 in presence of IFNγ. 2018. *Appl Microbiol Biotechnol* 102: 8895–8907.
20. Zhang, J., Zhang, Y., Li, Z., Liu, J., Shao, X., Wu, C., Wang, Y., Wang, K., Li, T., Liu, L., Chen, C., and Zhang, H. Outer membrane protein 25 of Brucella activates mitogen-activated protein kinase signal pathway in human trophoblast cells. 2017. *Front Vet Sci* 4: 197.
21. Tautermann, C.S., F. Binder, F.H. Büttner, C. Eickmeier, D. Fiegen, U. Gross, M.A. Grundl, R. Heilker, S. Hobson, S. Hoerer, A. Luippold, V. Mack, F. Montel, S. Peters, S. Bhattacharya, N. Vaidehi, G. Schnapp, S. Thamm, and M. Zeeb. 2019. Allosteric activation of striatal-enriched protein tyrosine phosphatase (STEP, PTPN5) by a fragment-like molecule. *Journal of Medicinal Chemistry* 62: 306–316.
22. Katayama, K., M. Yamaguchi, K. Noguchi, and Y. Sugimoto. 2014. Protein phosphatase complex PP5/PPP2R3C dephosphorylates P-glycoprotein/ABCB1 and down-regulates the expression and function. *Cancer Letters* 345: 124–131.
23. Kim, K.H., An, D.R., Song, J., Yoon, J.Y., Kim, H.S., Yoon, H.J., Im, H.N., Kim, J., Kim, D.J., Lee, S.J., Kim, K.H., Lee, H.M., Kim, H.J., Jo, E.K., Lee, J.Y., and Suh, S.W. Mycobacterium tuberculosis Eis protein initiates suppression of host immune responses by

acetylation of DUSP16/MKP-7. 2012. Proceedings of the National Academy of Sciences of the United States of America 109: 7729–7734.

24. Ding, H., Zheng, S., Garcia-Ruiz, D., Hou, D., Wei, Z., Liao, Z., Li, L., Zhang, Y., Han, X., Zen, K., Zhang, C.Y., Li, J., and Jiang, X. Fasting induces a subcutaneous-to-visceral fat switch mediated by microRNA-149-3p and suppression of PRDM16. 2016. *Nat Commun* 7: 11533.

25. Yu, L., Q. Liao, X. Chen, L. Xu, X. Zeng, Z. Lv, X. Sun, H. Zhen, and Z. Wu. 2014. Dynamic expression of miR-132, miR-212, and miR-146 in the brain of different hosts infected with Angiostrongylus cantonensis. *Parasitology Research* 113: 91–99.

PUBLISHER'S NOTE