Exosomal tpi-miR-10a-5p from *T. pisiformis* cysticerci regulates the expression of inflammatory factors in rabbits by targeting MAP3K7

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Abstract

Taenia pisiformis (*T. pisiformis*) cysticerci, belonging to Taeniidae, attaches to the wall of the mesentery and omentum in rabbits, causing cysticercosis pisiformis that can seriously affect the healthy development of the rabbit breeding industry. Helminths can produce exosomes, small vesicles containing proteins and RNAs. In our previous study, tpi-miR-10a-5p was found to be highly enriched in the exosomes from *T. pisiformis* cysticerci. In this study, we report that tpi-miR-10a-5p is significantly up-regulated in the blood and peripheral blood lymphocytes (PBLCs) in rabbits infected with *T. pisiformis* cysticerci. Furthermore, tpi-miR-10a-5p targets mitogen-activated protein kinase kinase kinase 7 (MAP3K7), the key gene involved in the c-Jun N-terminal kinase (JNK) signaling pathway. Knockdown of MAP3K7 inhibits the JNK signaling pathway, suppressing the production of inflammatory cytokines such as IFN-γ and TNFα, while overexpression of MAP3K7 activates the JNK signaling pathway in PBLCs. The same trend is observed with knockdown of

MAP3K7 when PBLCs were treated with exosomes from *T. pisiformis* cysticerci. *In vivo* experiments further demonstrate that the expression of MAP3K7, JNK, p-JNK, IFN-γ, and TNFα is significantly decreased in PBLCs during *T. pisiformis* infection. Therefore, tpi-miR-10a-5p can suppress the JNK signaling pathway and inflammatory response by targeting MAP3K7 in PBLCs. These findings may imply a mechanism used by the parasites releasing exosomes to sense and adapt to the host environment by regulating the immune reaction.

Keywords: *T. pisiformis* cysticerci; tpi-miR-10a-5p; MAP3K7; Exsome; Rabbits

1 Introduction

Cysticercosis pisiformis, caused by the larva of *Taenia pisiformis* (*T. pisiformis*), can lead to significant economic losses due to the poor physical condition of rabbits, such as weight loss (Yang et al., 2013), decreased prolificacy, and even death (Hallal-Calleros et al., 2016). The oncospheres hatch from the eggs after *T. pisiformis* infection and then migrated from the blood to the liver, and eventually to the abdominal cavity (Samorek-Pieróg et al., 2021). Lymphocytes in peripheral blood play a crucial role in maintaining immune response and resisting parasitic infections. A significant Th2-type responses is induced during *Schistosoma* infection due to cytokine production by peripheral blood mononuclear cells (Pearce and MacDonald, 2002). Studies have shown that parasite infection can alter the expression of miRNA and mRNA in peripheral blood lymphocytes (Lueong et al., 2013; Takeda et al., 2003). However, the specific role of peripheral blood lymphocytes in cysticercosis

pisiformis remains unclear.

Exosomes, extracellular vesicles with diameters ranging from 40 to 160 nm, play vital roles in regulating intercellular communication. They can release contents, including proteins and nucleic acids, by integrating with the cytomembrane or being taken up and internalized into phagosomes, particularly in the immune system (Kalluri and LeBleu, 2020; Pegtel and Gould, 2019). Research has shown that parasite-derived exosomes could be taken up by host cells to modulate immune response, which is beneficial for parasites to adapt to the host environment (Samoil et al., 2018). Many research demonstrated the presence of EVs in helminths, including flatworms, highlighting their potential role in intercellular communication and immune regulation. MicroRNAs (miRNAs), about 18-25 nucleotides in length, can regulate gene expression through binding and degrading the target messenger RNA (mRNA) (Lu and Rothenberg, 2018; Correia de Sousa et al., 2019). Recently, miRNAs have been identified in exosomes, which can modulate the function of recipient cells (Yu et al., 2016; Li et al., 2020; Zhang et al., 2015; Valadi et al., 2007). The exosomes from *Heligmosomoides polygyrus* containing miRNAs were transferred to mouse small intestinal epithelial cells and suppressed Type 2 innate responses (Buck et al., 2014). Exosome-like vesicles isolated from *Brugiamalayi* stimulated a classically activated macrophage differentiation in the J774A.1 cell line (Zamanian et al., 2015). The egr-miR-277a-3p of *Echinococcus granulosus* targeting NF-kB1 induced the production of pro-inflammatory cytokines and modulated the host immune responses (Zhang et al., 2022). Some studies have shown that helminth-derived exosomal

89 miRNAs can influence the host's inflammatory response (Bernal et al., 2014; Gracias
90 and Katsikis, 2011).
91 A previous study reported that miR-10a-5p is highly enriched in the exosomes from *T.*
92 *pisiformis* cysticerci (Wang et al., 2020). Therefore, the aim of this study is to clarify
93 the mechanism of *T. pisiformis* cysticerci-derived miR-10a-5p in exosomes regulating
94 PBLCs immune responses. This finding will demonstrate the function of *T. pisiformis*
95 cysticerci-derived miR-10a-5p in regulating host cell immune responses, and its
96 possible role in *T. pisiformis* immune evasion from host attacks, providing reliable
97 evidence for further investigation into the mechanism of *T. pisiformis* invasion.
98

99 # 2 Materials and methods

100 ## 2.1 Ethics

101 All animal experiment procedures were approved by the Animal Administration and
102 Ethics Committee of the Lanzhou Veterinary Research Institute, Chinese Academy of
103 Agricultural Sciences (LVRIAEC2021-028) and conducted in accordance with the
104 Guide for the Care and Use of Laboratory Animals of the Ministry of Science and
105 Technology of the People's Republic of China.
106

107 ## 2.2 Animals and parasites

108 *T. pisiformis* eggs were obtained from dogs experimentally infected with *T. pisiformis*
109 cysticerci and were maintained at the animal facilities of the Lanzhou Veterinary
110 Research Institute. New Zealand white rabbits weighing 1.5 to 2 kg (n = 12) were

purchased from the Laboratory Animal Center of the Lanzhou Veterinary Research Institute. Each rabbit in the experimental group (E, n=8) was artificially challenged with 1000 eggs of *T. pisiformis*, while the control group (C, n=4) was treated with the same volume of PBS (Phosphate Buffered Saline).

2.3 Cysticerci culture and collection of exosomes (EXO)

Cysticerci were harvested from the peritoneal cavities of rabbits infected with *T. pisiformis* and washed three times in sterile PBS supplemented with 100 μg/mL streptomycin and 100 IU/mL penicillin (Gibco, USA). Then the cysticerci were rinsed in RPMI 1640 medium and cultured in this medium with 10% exosome-depleted fetal bovine serum (FBS), 100 μg/mL streptomycin and 100 IU/mL penicillin in an incubator containing 5% CO_2 at 37 ℃. The culture medium was replaced after 12 h, and excretory/secretory products were collected at 24 h and 48 h.

A total of 100 mL of pooled excretory/secretory products were used to isolate the exosomes. Firstly, they were centrifuged for 10 min at 300 × g at 4 ℃ and then centrifuged for 30 min at 10,000 × g at 4 ℃ to eliminate cell fragments and dead cells. The supernatants were ultra-centrifuged for 90 min at 75,000 × g at 4 ℃ in a Beckman Coulter Optima L-100 XP centrifuge to remove large vesicles. Subsequently, the supernatants were sterilized using a 0.22 μm filter and further ultra-centrifuged for 90 min at 110,000 × g at 4 ℃ (Wang et al., 2020). The precipitate was washed with sterile PBS, then ultra-centrifuged at 110,000 × g for another 90 min. The exosomes were then re-suspended in 50 μL of PBS. Finally, the

protein concentration of the exosomes was determined with a BCA kit (Vazyme, China), and the amount of endotoxin concentration detected by the ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (GenScript, USA) was confirmed to be < 0.05 EU/ml. The exosomes were divided into aliquots and stored at -80 ℃.

2.4 Transmission electron microscope and nanoparticle tracking analysis (NTA)

To clarify the size, shape, and structure of exosomes, a microscopy technique called transmission electron microscopy (TEM) (Hitachi Ltd., Japan) was performed. 10 μL of exosomes were added to a 200-mesh copper grid and incubated for 10 min at room temperature. The morphology and size of the exosomes were observed under a transmission electron microscope at 80 kV after negative staining with 3% phosphotungstic acid (Sigma, USA).

Nanoparticle tracking analysis, which tracks the Brownian motion of each nanoparticle in a solution, was used to observe the size distribution of exosomes. This was done using a NanoSight LM10 instrument (Nanosight, UK) as previously reported (Tiwari et al., 2021).

2.5 Cells culture and treatment

About 30 mL of fresh peripheral blood from uninfected rabbits was collected in blood collection tubes with EDTA anticoagulant, and primary PBLCs were isolated from the fresh blood using the rabbit peripheral blood lymphocytes isolation kit (TBD Science,

China). The PBLCs were then plated into 12-well plates and cultured in RPMI-1640 medium with 10% FBS and 1% penicillin-streptomycin in an incubator witn 5% CO_2 at 37℃.

For transfection experiments, the RPMI-1640 medium was replaced with Opti-MEM (Invitrogen, USA). The PBLCs were transfected with 100 nmol/L of miR-10a-5p mimics or 100 nmol/L of mimics NC (negative control). For MAP3K7 silencing, the PBLCs were transfected with MAP3K7 (Gene ID: 100343571) siRNA or siRNA-NC (negative control, Sangon, China) using Lipofectamine™ RNAiMAX Transfection Reagent (Invitrogen, USA). In the MAP3K7 overexpression experiment, 2 μg/mL of pmCherry-N1 (empty vector) or 2 μg/mL of pmCherry-N1-MAP3K7 (Sangon, China) was transfected into the PBLCs using Lipofectamine 3000 (Invitrogen, USA). In the exosomes treatment experiments, PBLCs were treated with 25 μg/mL of exosomes or the same volume of PBS and then incubated at 37 ℃ for 24 h in an incubator. Each treatment was repeated three times. The sequences of all mimics and siRNAs are shown in Supplementary Table S1.

2.6 PCR and quantitative real-time PCR

Total RNA was extracted separately from *T. pisiformis* cysticercus, exosomes, PBLCs and fresh whole blood using TRIzol reagents (Invitrogen, USA). Nanodrop spectrophotometer (Thermo, USA) was used for RNA purity and quantification analysis. 1 μg of total RNA was reverse transcribed into cDNA using either the miRNA 1st Strand cDNA Synthesis Kit (by tailing A) or the HiScriptIII 1st Strand

cDNA Synthesis Kit (with＋gDNA wiper).

PCR was performed using 2 × Taq Master Mix on a Cycler (Thermo, USA). The temperature program consisted of an initial denaturation step at 94 ℃ for 5 min, followed by 34 cycles of denaturation at 94 ℃ for 30 sec, annealing at 58 ℃ for 30 sec, and extension at 72 ℃ for 6 sec, with a final extension step at 72 ℃ for 7 min. PCR amplification products were detected by agarose gel electrophoresis.

qPCR was performed on an ABI 7500 instrument (Thermo, USA) using the SYBR Green Premix Pro Taq HS qPCR Kit. The protocol included initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 10 sec, and annealing/extension at 60 °C for 30 sec. The relative expression levels of mRNA/miRNA were normalized to GAPDH/U6 and calculated using the $2^{-\Delta\Delta Ct}$. Statistical analysis was based on data from three independent experiments. The primer sequences can be found in Table 1.

2.7 Luciferase assay

1×10^5 HEK293T cells were seeded per well on 24-well plates. 1.5 μg of pmirGLO-MAP3K7-WT (MAP3K7-WT, Sangon, China) or pmirGLO-MAP3K7-Mut (MAP3K7-Mut, Sangon, China) was co-transfected with 20 μmol/L of miR-10a-5p mimics or mimics NC using Lipofectamine 3000 when cells reached 70~80% confluence. The cells were then incubated for 24 h in a 5% CO_2 incubator at 37 ℃. Luciferase activity in the supernatant of the lysed cells was measured using a Dual Luciferase Reporter Assay System (Vazyme, China), and the

ratio of firefly luciferase activity to that of Renilla was calculated.

2.8 Western blot analysis

Total protein was extracted from cells using RIPA buffer (Thermo, USA) supplemented with protease-phosphatase inhibitor (NCM Biotech, China) and quantified using BCA kit (Vazyme, China). Approximately 25 μg of protein was separated by 12% SDS polyacrylamide gel electrophoresis and transferred to PVDF membranes. After blocking with 5% non-fat dried milk, the PVDF membranes were incubated with corresponding primary antibodies at 4 °C overnight: rabbit anti-MAP3K7 polyclonal antibody (1:1000, Affinity, China), rabbit anti-JNK polyclonal antibody (1:1000, Affinity, China), rabbit anti-p-JNK polyclonal antibody (1:1000, Affinity, China), rabbit anti-IFN-γ polyclonal antibody (1:1000, Affinity, China), mouse anti-TNFα monoclonal antibody (1:1000, Proteintech, China), rabbit anti-IL10 polyclonal antibody (1:1000, Affinity, China) and rabbit anti-β-actin polyclonal antibody (1:5000, Bioss, China). After washing three times with Tris-buffered saline with 0.1% Tween 20 (TBST), the membranes were subsequently incubated with rabbit anti-mouse IgG-HRP antibody (1:4000, Biodragon, China) or goat anti-rabbit IgG-HRP antibody (1:4000, Biodragon, China) for 1h at room temperature. The bands were visualized using high resolution image acquisition system (BioRad, USA) with BeyoECL Moon reagent (Beyotime, China), and the intensities of the bands were analyzed using ImageJ software.

2.9 Statistical analysis

Statistical analysis was conducted using with GraphPad Prism 8. A *t*-test was used to compare differences between two groups, with a significant level set at $p < 0.05$.

3 Results

3.1 Release of tpi-miR-10a-5p derived from exosomes of *T. pisiformis* cysticerci into the rabbit blood stream

NTA and TEM showed that the exosomes were round or oval vesicles with a diameter of approximately 30 ~ 140 nm, as expected (Figure 1A, 1B). The tpi-miR-10a-5p sequence was amplified by RT-PCR using total RNA from exosomes that were reversely transcribed to cDNA via the miRNA 1st Strand cDNA Synthesis Kit (with A-tailing) as a template (Figure 1C), and further confirmed by sequencing (Figure S1). At same time, the precursor sequence of tpi-miR-10a-5p was amplified from *T. pisiformis* cysticerci, and confirmed by sequencing (Figure S2), but no results were found from rabbits, only some primer dimers (Figure 1D). The expression level of tpi-miR-10a-5p was significantly up-regulated in the blood and PBLCs of rabbits infected with *T. pisiformis* (Figure 1E, 1F). In summary, the above results demonstrate that tpi-miR-10a-5p was potentially released into the blood stream of rabbits by exosomes of *T. pisiformis* cysticerci.

Fig. 1 | Tpi-miR-10a-5p exists in exosomes and rabbits infected with *T. pisiformis*.

(A) Nanoparticle tracking analysis of exosomes. **(B)** Morphology of exosomes

observed by transmission electron microscope. **(C)** PCR amplification products of tpi-miR-10a-5p from exosomes. M: DNA maker; 1~2: PCR products from exosomes; 3: Negative control. **(D)** PCR amplification products of the precursor sequence of tpi-miR-10a-5p from *T. pisiformis* cysticerci or rabbits. M: DNA maker; 1: PCR products from PBLCs of rabbits; 2: PCR products from *T. pisiformis* cysticerci; 3: Negative control. **(E)** The relative expression level of tpi-miR-10a-5p in rabbit blood was determined by RT-qPCR. **(F)** The relative expression level of tpi-miR-10a-5p in rabbit PBLCs was determined by RT-qPCR. The corresponding *p* value was calculated between the experimental group and the control group by *t*-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Abbreviations: 30d-C: PBLCs from rabbits 30 days treated with PBS; 30d-E: PBLCs from rabbits 30 days post-infection with *T. pisiformis cysticerci.*

3.2 Tpi-miR-10a-5 suppresses MAP3K7 expression by directly binding to the 3'-UTR

Initially, MAP3K7 was identified as a candidate target gene of tpi-miR-10a-5p through predictions from the miRDB and TargetScan databases, and was further validated by RT-qPCR. MAP3K7, a gene associated with immune response, was significantly down-regulated in tpi-miR-10a-5p overexpressed PBLCs (Figure 2A). Subsequent analysis revealed that the 3′-UTR of MAP3K7 contained a potential binding site for tpi-miR-10a-5p (Figure 2B). Additionally, analysis of MAP3K7 levels, both mRNA and protein, indicated that tpi-miR-10a-5 overexpression can

inhibit MAP3K7 expression in PBLCs (Figure 2C, 2D, 2E). Further validation of the relationship between tpi-miR-10a-5p and MAP3K7 was conducted, showing that luciferase activity decreased in HEK293T cells co-transfected with pmirGLO-MAP3K7-WT and tpi-miR-10a-5p mimics (Figure 2F). Furthermore, MAP3K7 expression was reduced in PBLCs from rabbits 30 days post-infection (Figure 2G). The correlation between miR-10-5p and MAP3K7 relative expression in infected and uninfected PBLCs was analyzed and the correlation coefficient (R) was -0.5299, indicating a negative correlation between the two variables (Figure S3). These results confirm that the expression of MAP3K7 can be regulated by tpi-miR-10a-5p derived from *T. pisiformis.*

Fig. 2 | MAP3K7 is a direct target of tpi-miR-10a-5p. (A) The expression levels of candidate target genes in PBLCs transfected with tpi-miR-10a-5p mimics were detected by RT-qPCR. **(B)** The potential binding site of tpi-miR-10a-5p and MAP3K7 3′-UTR, as well as the sequences of wild-type and mutant plasmids. **(C)** Transfection efficiency of tpi-miR-10a-5p mimics in PBLCs was detected by RT-qPCR. **(D)** The relative mRNA and **(E)** protein expression levels of MAP3K7 in tpi-miR-10a-5p-overexpressed PBLCs were detected by RT-qPCR and Western blot, respectively. **(F)** The activity of luciferase in HEK293T cells transfected with tpi-miR-10a-5p mimics and pmirGLO-MAP3K7-WT or pmirGLO-MAP3K7-Mut, respectively. **(G)** The expression level of MAP3K7 in PBLCs from 30 days *T. pisiformis* -infected rabbits was detected by RT-qPCR. The corresponding *p* value

was calculated between the experimental group and the control group by *t*-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: MAP3K7-WT: pmirGLO-MAP3K7-Wildtype; MAP3K7-Mut: pmirGLO-MAP3K7-Mutant; 30d-C: PBLCs from rabbit 30 days treated with PBS; 30d-E: PBLCs from rabbits 30 days post-infection with *T. pisiformis.*

3.3 Regulation of the JNK pathway and production of inflammatory cytokines by MAP3K7

The interference efficiency of different MAP3K7 siRNAs was compared (Figure 3A), and siMAP3K7-15, which had the highest interference efficiency, was used for the following experiments. The relative expression levels of IFN-γ, TNFα, and iNOS were significantly decreased in PBLCs transfected with MAP3K7 siRNA, while IL10 expression was increased (Figure 3B). Conversely, PBLCs transfected with pmCherry-N1-MAP3K7 showed the opposite results (Figure 3C). The protein levels of JNK, p-JNK, and IFN-γ were significantly down-regulated in PBLCs transfected with MAP3K7 siRNA, while their protein levels were significantly up-regulated in PBLCs transfected with pmCherry-N1-MAP3K7. The protein level of IL10 was up-regulated in PBLCs transfected with MAP3K7 siRNA, while the level of IL10 was down-regulated in PBLCs transfected with pmCherry-N1-MAP3K7 (Figure 3D).

Fig. 3 | MAP3K7 is a key regulator of the JNK pathway. (A) The expression level of MAP3K7 in PBLCs transfected with three different MAP3K7 siRNAs was

detected by RT-qPCR. **(B)** The expression levels of inflammatory cytokines in PBLCs transfected with MAP3K7 siRNA and **(C)** pmCherry-N1-MAP3K7 were detected by RT-qPCR. **(D)** Protein expression levels of JNK, p-JNK, IFN-γ and IL10 in PBLCs transfected with MAP3K7 siRNA and pmCherry-N1-MAP3K7 were detected by Western blot. The corresponding *p* value was calculated between the experimental group and the control group by *t*-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: siR-NC: siRNA-NC; siMAP3K7: MAP3K7 siRNA; EV: Empty Vector (pmCherry-N1); MAP3K7: pmCherry-N1-MAP3K7.

3.4 Production of pro-inflammatory cytokines is inhibited in PBLCs treated with exosomes and in rabbits infected with *T. pisiformis*

Real-time RT-PCR showed that the level of tpi-miR-10a-5p increased with increasing of exosomes concentration (Figure 4A), while the expression of MAP3K7 decreased in PBLCs treated with exosomes (Figure 4B). Additionally, the expressions level of IFN-γ, TNFα, and iNOS were significantly reduced in PBLCs treated with exosomes, while IL10 expression increased (Figure 4C). The protein levels of MAP3K7, JNK, p-JNK, TNFα, and IFN-γ were notably down-regulated in PBLCs treated with exosomes, while IL10 expression was up-regulated (Figure 4D). *In vivo*, only the expression of MAP3K7, JNK, p-JNK, IFN-γ, and TNFα were significantly decreased at 30 days post *T. pisiformis* infection, consistent with the results *in vitro* (Figure 4E, 4F).

Fig. 4 | Production of pro-inflammatory cytokines is inhibited in PBLCs treated with exosomes or infected with *T. pisiformis*. (A) The levels of miR-10a-5p, **(B)** MAP3K7 and **(C)** inflammatory cytokines in PBLCs treated with exosomes were analyzed. **(D)** Protein expression levels of MAP3K7, JNK, p-JNK, IFN-γ TNFα and IL10 in PBLCs treated with exosomes were detected using Western blot. **(E)** The expression levels of inflammatory cytokines in PBLCs from 30 days *T. pisiformis* -infected rabbits were detected by RT-qPCR. **(F)** Protein expression levels of MAP3K7, JNK, p-JNK, IFN-γ TNFα and IL10 in PBLCs from 30 days *T. pisiformis* -infected rabbits were detected by Western blot. The corresponding *p* value was calculated between the experimental and the control group by *t*-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: PBS: PBLCs treated with PBS; EXO: PBLCs treated with exosomes; 30d-C: PBLCs from rabbit 30 days treated with PBS; 30d-E: PBLCs from rabbit 30 days post-infection with *T. pisiformis.*

4 Discussion

Cysticercosis pisiformis, caused by the larval stage of *T. pisiformis*, is a common parasitic disease in rabbits. However, it remains unclear how *T. pisiformis* cysticerci modulates the immune response in the host, thereby promoting parasite invasion and survival within the host. Throughout the long-term evolution, the complex interaction between the parasite and the host has established a delicate balance conducive to the survival and development of both parties (Su et al., 2020).

Exosomes, small extracellular vesicles, are important carriers of material exchange containing proteins and RNAs. Immunocytes such as dendritic cells and macrophages can uptake exosomes derived from parasites, indirectly regulating the function of recipient cells (Eichenberger et al., 2018). The extracellular vesicles derived from *E. granulosus* hydatid fluid can modulate the immune response, leading to increased production of certain cytokines such as IL-10, and TNF-α in sheep PBMCs during infection (Yang et al., 2021). Exosomes from *Leishmania* can transfer proteins to host macrophages, thereby eliciting acquired immune responses (Silverman et al., 2010; Weber et al., 2023). Consequently, the immunoregulatory effects induced by exosomes can be attributed to the complex substances they carry, such as miRNAs and proteins. Exosomal miRNAs from *T. pisiformis* cysticerci are reported to regulate macrophage polarization and inhibit the immune response in rabbits (Wang et al., 2021; Chen et al., 2023). In our previous research, small RNA sequencing revealed that tpi-miR-10a-5p was enriched in exosomes derived from *T. pisiformis* cysticerci (Wang et al., 2020), but the role of tpi-miR-10a-5p in the interaction between cysticercus and rabbits is unclear. Tran et al demonstrated that one of the most abundant miRNAs, fhe-miR-125b, is released by the parasite via exosomes. This miRNA regulates the activation of pro-inflammatory M1 macrophages in BALB/c mice infected with *Fasciola hepatica* [30]. Based on the above results, we infer that *T. pisiformis* cysticerci-derived miR-10a-5p can be released into the bloodstream through exosome transportation and regulate the function of host cells. In this study, parasite-derived miR-10a-5p was identified to be expressed in both serum and PBLCs

375 in rabbits infected with *T. pisiformis*. Furthermore, the level of tpi-miR-10a-5p was
376 up-regulated in the PBLCs of rabbit infected *T. pisiformis*, displaying a closer
377 correlation between the level of tpi-miR-10a-5p and cysticercus infection, which
378 implies that the function of PBLCs can be regulated by serum tpi-miR-10a-5p uptake
379 in *T. pisiformis* infected rabbits.
380 *In vitro* and *in vivo* experiments have confirmed that tpi-miR-10a-5p can target
381 MAP3K7, which is associated with the activation of NF-kB, JNK and p38
382 (Ninomiya-Tsuji et al., 1999; Sun et al., 2022). The JNK and p38 pathway both
383 belonging to the MAPK (mitogen-activated protein kinases) family are well-known
384 for their involvement in the regulation of inflammatory reactions (Rincón et al., 2000).
385 Our experiments involving MAP3K7 knockdown and over-expression demonstrated
386 the impact of MAP3K7 expression on JNK, p-JNK, IFN-γ, and IL10 production,
387 which further indicated that MAP3K7 plays an important role in host immune
388 response.
389 Additionally, as exosome concentrations increased, the expression of tpi-miR-10a-5p
390 also increased in PBLCs, indicating that tpi-miR-10a-5p was enriched in *T. pisiformis*
391 cysticerci derived exosomes. Interestingly, the expression of the target gene MAP3K7
392 did not follow the same trend as tpi-miR-10a-5p overexpression after exosome
393 treatment, suggesting that MAP3K7 may be modulated by other *T. pisiformis*
394 cysticerci exosomal RNAs and proteins in addition to tpi-miR-10a-5p. Multiple
395 studies have shown that each gene can be regulated by several miRNAs, contributing
396 to a complex regulatory network. As a result, MAP3K7 exhibited a different

expression pattern between exosomes and tpi-miR-10a-5p treated PBLCs. Long non-coding RNA CCDC144NL-AS1 can act as a sponge for miR-143-3p and up-regulate MAP3K7 by competing as an endogenous RNA in gastric cancer, indicating that MAP3K7 is regulated not only by miR-143-3p but also by LncRNA. Further research will be conducted to elucidate the potential regulatory mechanism of MAP3K7 in rabbit PBLCs treated with *T. pisiformis* cysticerci exosomes.

The expression level of iNOS was substantially down-regulated in RAW264.7 cells treated with extracellular vesicles from *E. multilocularis* (Zheng et al., 2017). In our experiments, crucial genes involved in the JNK signaling pathway, such as JNK and p-JNK, were reduced in PBLCs treated with exosomes. Additionally, the expression of inflammatory cytokines like IFN-γ and TNFα was also decreased, while the expression of IL10 was increased. Previous studies have shown that miRNA from parasite exosomes can regulate host immune responses (Bernal et al., 2014), and our study also suggests that immune regulation may be partially attributed to the high enrichment of tpi-miR-10a-5p in exosomes. *In vivo* experiments, the expression of tpi-miR-10a-5p was increased and MAP3K7 was decreased in PBLCs from rabbits 30 days post-infection with *T. pisiformis.* At the same time, the expressions of JNK, p-JNK, IFN-γ, and TNFα were significantly decreased, and IL10 showed an increasing trend but was not significant. This suggests that the results in rabbits infected with *T. pisiformis* were in substantial agreement with those in the in *vitro* experiment.

The production of inflammatory cytokines, including pro- and anti-inflammatory

cytokines in the host, is influenced by various factors and regulatory networks. In the last stages of helminth infection, there is an increase in the production of anti-inflammatory cytokines, which is thought to be crucial for both eliminating parasites and repairing damaged tissues (Peng et al., 2022; Vacca and Le Gros, 2022). Exosomes, which are key components of parasites' excretory/secretory products, present a novel concept and method for communication between the parasite and host cells (Marcilla et al., 2012). Additionally, exosomal miRNA may have a significant impact on important role in regulating and evading the host immune response throughout the infection process.

5 Conclusions

C. pisiformis-derived tpi-miR-10a-5p can be released into the peripheral blood of rabbits through exosomes. As a result, the target gene of tpi-miR-10a-5p, MAP3K7, is down-regulated, leading to the suppression of inflammatory cytokines production through the JNK pathway, which is beneficial for the survival and development of *T. pisiformis* cysticerci in the rabbits.

Funding

This work was supported by the National Key Research and Development Program of China (2023YFD1802401), the National Natural Science Foundation of China (32072889), and the Science and Technology Program of the Department of Science and Technology of the Tibet Autonomous Region (XZ202401JD0012). We thank the

scientific staff at the Instrument Center of Lanzhou Veterinary Research Institute for their technical assistance.

Potential conflicts of interest

The authors have declared that no competing interests exist.

Author contributions

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Xueyong Zhang: Conceptualization, Supervision, Validation, Writing– review & editing

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Table 1 Primers for PCR and qPCR

Primer	Sequence（5’-3’）
pre-tpi-miR-10a-5p forward	CACCCTGTAGACCCGAGTTTGAG
pre-tpi-miR-10a-5p reverse	TGATGCCTTGAAGACACGAGC
tpi-miR-10a-5p forward	ATATATCACCCTGTAGACCCG
tpi-miR-10a-5p reverse	GCTGTCAACGATACGCTACG
MAP3K7 forward	GAGGAGCCTTTGGAGTGGTT

MAP3K7 reverse	ACTGCCGAAGCTCCACAATA
IFN-γ forward	GGCTTTATACCTGGGGCCAAAT
IFN-γ reverse	AGCAGTGGCTCAGAATGCAG
TNFα forward	CGTAGTAGCAAACCCGCAAGTG
TNFα reverse	CGCTGAAGAGAACCTGGGAGTAG
iNOS forward	GTTCAGAAGGGAGTAACCGCT
iNOS reverse	GGCCTAGGAAAGAATGTGAGA
IL10 forward	GTCACCGATTTCTCCCCTGT
IL10 reverse	GATGTCAAACTCACTCATGGCT
GAPDH forward	TTGAAGGGCGGAGCCAAAA
GAPDH reverse	CAGGATGCGTTGCTGACAATC
MAP4K1 forward	CGAGATGAGCACCGAGCACAAG
MAP4K1 reverse	CGAGATGAGCACCGAGCACAAG
ASNS forward	TTCATCGGTTGGCAGTGGTTGAC
ASNS reverse	TCGCCTTGTGGTTGTAGATTTCTCC
NFATC2 forward	CCGCTGGAGCCCAAGAACAAC
NFATC2 reverse	TCCTGCCGATGTCCGTCTCAC
HOXB3 forward	TTCATCCTTCTCTACCCTGCTCCTC
HOXB3 reverse	CGATGAGACGGGTGTGGAAGTTAAG
NACA forward	CTCGGACTGCCTTTGCTCTTGAC
NACA reverse	GCCCCAAGCCCTACGAGTCTG
ATF4 forward	GCCGTCTTGTTCTGCTCCATCTTC

ATF4 reverse	TCTCTGTCTGCTCTCCTTGCTACC
GATA3 forward	GCTCTTACAGTGCCGAGAAACCC
GATA3 reverse	CAGCAGCTTCTACCTGGACA
RORA forward	GCCTGATGCTGGTGTGTAGT
RORA reverse	GGCTTTATACCTGGGGCCAAAT

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