Expression profiling of circulating miRNAs in mouse serum in response to *Echinococcus multilocularis* infection

XIAOLA GUO[1]* *and* YADONG ZHENG[1,2]*

[1] *State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, CAAS, Lanzhou 730046, Gansu, China*
[2] *Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, China*

(*Received 15 December 2016; revised 9 February 2017; accepted 12 February 2017; first published online 8 March 2017*)

SUMMARY

Echinococcus multilocularis is a most pathogenic zoonotic tapeworm that causes devastating echinococcosis in both humans and animals. Circulating microRNAs (miRNAs) are stably existed in the serum/plasma of mammalian hosts during helminthic infection. In this study, we compared the host-circulating miRNA expression in the sera from the *E. multilocularis*-infected and uninfected mice. A total of 58 host-origin serum miRNAs were differentially expressed (2 ⩾ fold change, $P < 0{\cdot}05$), of which 21 were upregulated and 37 were significantly downregulated. Consistent with the sequencing data, quantitative polymerase chain reaction (PCR) results showed that the expression levels of four miRNAs were elevated gradually and one decreased gradually at the *E. multilocularis* infection time points. Moreover, seven of *E. multilocularis* specific miRNAs were identified in the sera. Real-time PCR analyses further demonstrated that only two parasite-derived miRNAs (emu-miR-10 and emu-miR-227) were specifically amplified in all the sera from mice infected with *E. multilocularis*. These findings will be helpful to understand the roles of miRNAs in host–parasite interaction and to potentiate serum miRNAs as diagnostic targets for echinococcosis.

Key words: microRNAs, serum, *Echinococcus multilocularis*.

INTRODUCTION

Alveolar echinococcosis (AE) is a zoonosis with great public health concerns, caused by a tapeworm *Echinococcus multilocularis* (Deplazes and Eckert, 2001). AE is mainly distributed in the northern hemisphere, in particular regions of China and the Russian Federation and countries in continental Europe and North America (Craig *et al*. 2007). AE is characterized by a tumour-like growth of *E. multilocularis* metacestodes in the liver of rodents or human beings (Czermak *et al*. 2008; Zhang *et al*. 2012). It is estimated that approximately 0·3–0·5 million people are suffering from AE worldwide (Zhang *et al*. 2015). The estimated worldwide human burden of AE were 650 000 disability-adjusted life years annually, with most of the disease burden concentrated in western China (Torgerson *et al*. 2010). The limited availability of diagnostics is considered to be one of contributing factors for prevalence of echinococcosis (Wang *et al*. 2013). Parasite-specific antibodies are not be detected during the early stages of infection (Yamano *et al*. 2014). Therefore, ideal markers with high specificity and high sensitivity for early and specific diagnosis of *E. multilocularis* are urgently needed.

microRNAs (miRNAs), a class of short endogenous non-coding RNAs, regulate the gene expression. Accumulating studies have shown that miRNAs are key regulators in physiological and pathological processes, such as immune response, inflammatory response and tumour occurrence (Baltimore *et al*. 2008; Sonkoly and Pivarcsi, 2009). Using deep sequencing and miRNA microarray, host miRNAs in the relevant cells or tissues have been shown to be dysregulated during parasite infection, demonstrating their important roles in host responses to pathogen challenge (Xu *et al*. 2013; Zheng *et al*. 2013). Functional prediction revealed that some of them were involved in the immune and inflammatory response to parasitic infection (Judice *et al*. 2016). Recent studies showed that circulating miRNAs can be stably detectable in blood or fluids of humans and animals with helminth infection (Cheng *et al*. 2013; Xu *et al*. 2013; Hoy *et al*. 2014) and have been extensively studied because of the potential use as diagnostic biomarkers for the early detection of parasite infection (Hoy *et al*. 2014; Jia *et al*. 2014). For example, the host-circulating sja-miR-223 has a potential biomarker for *Shistosoma japonicum* infection (Wang *et al*. 2010; He *et al*. 2013). Recent study showed that ovi-miR-192 has the potential to be used as a diagnostic biomarker

* Corresponding authors: State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, CAAS, 1 Xujiaping, Lanzhou 730046, Gansu, China. E-mail: guoxiaola@caas.cn or zhengyadong@caas.cn

Parasitology (2017), **144**, 1079–1087.
doi:10.1017/S0031182017000300

for *Opisthorchis viverrin*-induced cholangiocarcinoma (Silakit *et al*. 2014). Moreover, accumulating studies have showed that parasite-derived circulating miRNAs can be detected in the serum/plasma of the hosts and served as specific biomarkers for early detection of helminthic infections (Cai *et al*. 2016).

Echinococcus species miRNAs have recently been described (Cucher *et al*. 2011, 2015), providing a possibility of understanding their roles in development and growth, host–parasite interaction, and their future potential use as diagnostic targets. However, circulating miRNA expression associated with *E. multilocularis* infections have not been profiled. In the study, we demonstrate that several hostcirculating miRNAs are dysregulated in the mouse sera during *E. multilocularis* infection. We also identify seven parasite-derived miRNAs in the sera of mice infected with *E. multilocularis*. The results will help us to further understand the molecular basis for host–parasite interactions and to potentiate parasite-derived miRNAs in host serum as a diagnostic target of parasite infection.

MATERIALS AND METHODS

Ethics statement

All animal experiments in the study were handled in strict accordance with good animal practice according to the Animal Ethics Procedures and Guidelines of the People's Republic of China (Regulations for Administration of Affairs Concerning Experimental Animals, China, 1988). All animal experiments in the study were approved by Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, and were conducted strictly according to the guidelines.

Parasite infections and serum collection

Echinococcus multilocularis Qinghai isolate (Chinese mainland strain, Qinghai population) was maintained in DBA/2 mice in our laboratory. Thirty female DBA/2 mice (6-week-old) were randomly divided into two groups. For blood collection from mice infected with *E. multilocularis*, one groups of DBA/2 were peritoneally infected with approximately 1000 protoscolecesper mouse. One groups inoculated with phosphate buffer saline (PBS) were used as control. The mice were exsanguinated at different time points after *E. multilocularis* infection (4, 8 and 12-week post-infection). Serum was prepared and immediately stored at −80 °C prior to RNA extraction.

High-throughput sequencing

Total RNA was extracted from the sera of one healthy mouse and one mouse infected with *E. multilocularis* at 4-week post-infection using miRNAesy Serum/Plasma Kit (Qiagen, Germany), respectively. Small RNA sequencing was carried out in BGI-Shenzhen (Shenzhen, China). For construction of sequencing libraries, total RNA was subjected to polyacrylamide gel electrophoresis (PAGE) and small RNAs with a size of 18–30 nt were recovered (Jin *et al*. 2017). Recovered small RNAs were added with 5′ and 3′ adaptors, respectively, followed by polymerase chain reaction (PCR) enrichment. PCR products were purified by using the PAGE gels and the fraction ranging from 62 to 75 nt was excised, eluted and analysed by Bioanalyser (Agilent). After RNA sequencing, we performed the following bioinformatical analysis according to previously described methods (Cheng *et al*. 2013). Briefly, after removing low-quality reads and adaptor sequences, only sequences perfectly matched the mouse genome (http://www.ncbi.nlm.nih.gov/genome/genomes/52) were used for the following analysis. Mouse known miRNAs were identified by BLAST searching against the MirGeneDB database (http://www.mirgenedb.org/index.shtml, release 21) (Cheng *et al*. 2013) and the relative miRNA expression levels were analysed using DEGseq method.

To identify *E. multilocularis*-specific miRNA in the serum of *E. multilocularis*-infected mouse, we performed the following analyses: small RNAs in the two libraries that were mapped perfectly to the mouse genome were filtered using the miRDeep program (Cucher *et al*. 2015) and the rest were used for further analysis. Only small RNAs were uniquely mapped to the *E. multilocularis* genome (ftp://ftp.sanger.ac.uk/pub/pathogens/Echinococcus) were retained and taken for further analysed against miRbase for determining the known miRNA.

Targets of differentially expressed miRNAs were predicted using the online software TargetScan (http://www.targetscan.org/vert_71) (Agarwal *et al*. 2015), miRanda (http://www.microrna.org/microrna) (Betel *et al*. 2008) and RNAhybrid (Lai and Meyer, 2016). The target genes were analysed in terms of Gene Ontology (GO) categories and KEGG pathway enrichment analyses using the Database for Annotation, Visualization and Integrated Discovery (DAVID) gene annotation tool (Dennis *et al*. 2003).

Quantitative real-time PCR (qRT–PCR) and a stem-loop-based PCR

A miScript II RT Kit (Qiagen) was used to reversely transcribe RNA to cDNA. The 20 μL reverse transcription reaction contained 2 μL total RNA (approximate 50 ng), 2·2 μL Plasma Spike-In Control (10^8copies μL^{-1}), 5 μL 5 × miScript HiFlex buffer, 2 μL 10 × miScript Nucleics Mix, 2 μL miScript Reverse Transcriptase Mix and 7·8 μL RNase-free water. The temperature program was

https://doi.org/10.1017/S0031182017000300 Published online by Cambridge University Press

60 min at 37 °C, 5 min at 95 °C and then held at 4 °C. RT–PCR was performed using miScript SYBR Green PCR Kit (Qiagen). The 20 μL PCR reaction included 2 μL of cDNA (1:5 dilution), 10 μL 2 × QuantiTect SYBR Green PCR Master Mix, 2 μL 10 × miScript Universal Primer, 2 μL 10 × miScript Primer Assay and 4 μL H_2O. The reactions were conducted in a 96-well plate at 95 °C for 15 min, followed by 40 cycles of 94 °C for 15 s, 55 °C for 30 s and 70 °C for 30 s. The miScript Primers were listed in online Supplementary Table S1. *Ct* values were median-normalized to synthetic spike – in *Caenorhabditis elegans*-miR-39 (cel-miR-39) as described previously (Mitchell *et al*. 2008). Relative fold change was calculated as $2^{-\Delta Ctn}$, where *Ctn* stands for normalized *Ct* values (Mitchell *et al*. 2008; Hoy *et al*. 2014).

For analysis of *E. multilocularis*-specific miRNA in the sera, the miRNA LNA™ PCR primers were ordered from Exiqon (online Supplementary Table S1). Reverse transcription reactions were performed using miRCURY LNA™ Universal cDNA Synthesis Kit II (Exiqon) according to the manufacturer's protocol. PCR was carried out with miRCURY LNA™ SYBR Green master mix (Exiqon). The fold changes were calculated as the ratio of normalized values in infected serum compared with the background in the uninfected serum.

To examine *E. multilocularis*-specific miRNA in the infected sera, seven miRNAs were chosen for assay by a stem-loop-based PCR using total RNA from *E. multilocularis*-infected mouse sera (Salone and Rederstorff, 2015). The stem-loop-based PCR primers were listed in online Supplementary Table S2. First, 50 ng of serum RNA was input for first-strand cDNA synthesis (ThermoFisher Scientific). PCR was conducted with the following steps: 95 °C for 10 min, 40 cycles of 96 °C for 5 s, 54 °C for 5 s and 68 °C for 3 s, and then 72 °C for 1 min. PCR products were resolved on 2% agarose gel, purified and sequenced (Takara, Japan).

Statistical analysis

Data analyses were performed using GraphPad Prism 5 software (Version 5.00 for Windows, GraphPad Software, San Diego, California USA) and a one-tailed unpaired *t*-test was used for comparison of two groups, with a P value <0·05 being considered to be significantly different.

RESULTS

Sequencing of circulating miRNAs in the serum of E. multilocularis*-infected mouse*

To identify host-circulating miRNAs of which the expression is altered upon *E. multilocularis* infection, the small RNAs in the sera from the infected and healthy mice were sequenced. In this study, 23 584 745 and 23 900 492 total reads were obtained from the uninfected and *E. multilocularis*-infected libraries, respectively (online Supplementary Table S3). After removing low-quality reads and adapter sequences, a total of 23, 244, 523 and 23, 497, 669 clean reads were ultimately obtained (online Supplementary Table S3). The size distribution of small RNA reads was similar between the two libraries, and the majority of these miRNA were 21–23 nt in length (Fig. 1A). These results indicated the small RNA libraries were highly enriched in miRNA sequences. In addition to miRNAs, a series of other small RNAs (sRNAs) including rRNAs, tRNAs, snRNAs and snoRNAs, were also detected in two samples (online Supplementary Fig. S1). Both rRNAs and tRNAs accounted for very high proportions in the sequenced reads (online Supplementary Fig. S1).

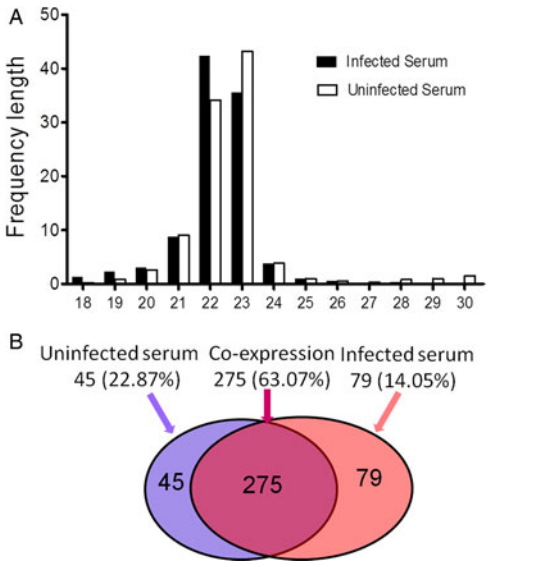

Fig. 1. Size distribution of small RNA sequences and unique miRNAs in two libraries. (A) The size distribution of the small RNA sequences in the sera from the *Echinococcus multilocularis*-infected and uninfected mice; (B) The distribution of 399 unique miRNAs identified in both uninfected (left, blue circle) and infected mouse sera (right, red circle). The overlapping region indicates co-expressed unique miRNAs.

Identification of host-circulating miRNAs differentially expressed in the serum of E. multilocularis*-infected mouse*

A total of 355 and 321 known miRNAs was identified in the uninfected and *E. multilocularis*-infected libraries, respectively (Table 1), and a total of 399 unique miRNAs were obtained from both libraries. As shown in Fig. 1B, 275 of 399 unique miRNAs (63·07%) were co-expressed in both libraries, while 79 (14·05%) and 45 (22·87%) were exclusively expressed in the infected and uninfected mouse,

https://doi.org/10.1017/S0031182017000300 Published online by Cambridge University Press

Table 1. Summary of known miRNA in each library

	Known miRNA	Infected serum	Uninfected serum
miRNA		355	321
miRNA-5p	788	162	153
miRNA-3p	796	167	148
miRNA precursors	1186	310	283
Unique precursors matched to miRNA precursors	–	1470	1338
Total precursors matched to miRNA precursors	–	12 904 613	12 193 503

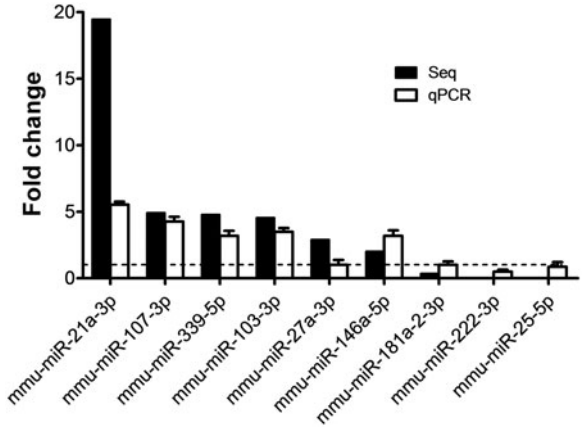

Fig. 2. Validation of miRNA expression by qPCR in the sera of mice infected with *Echinococcus multilocularis* 4-week post-infection. Data for the final analysis were from three independent experiments. The dashed horizontal line indicates the relative miRNA expression in each uninfected sera, which was set as 1·0.

respectively. Moreover, 58 of 399 miRNAs were significantly differentially expressed (fold change ⩾2 and *P*-value <0·05), of which 21 were upregulated and 37 were significantly downregulated. The detail of miRNA expression data was listed in online Supplementary Table S4. Among these 58 miRNAs, 8 and 18 were specifically expressed in the sera of *E. multilocularis*-infected and uninfected mouse, and 32 were shared in both libraries.

Validation of differentially expressed miRNAs by qPCR analysis

To further validate sequencing data, nine differentially expressed miRNAs were randomly selected and their expression was analysed by qPCR. Consistent with the sequencing results, there was an increase in expression of mmu-miR-103-3p, mmu-miR-107-3p, mmu-miR-339-5p, mmu-miR-146a-5p and mmu-miR-21a-3p, and a reduction in expression of mmu-miR-222-3p in the *E. Multilocularis*-infected mouse sera compared with the control (Fig. 2). However, mmu-miR-27a-3p, mmu-miR-25-5p and mmu-miR-181a-2-3p did not show different expressions and were not analysed further (online Supplementary Table S5).

Dynamic relative expression of six miRNAs in the mouse sera during E. multilocularis *infection by qPCR*

For the 6 miRNAs validated to be differently expressed at 4 weeks post-infection, we next examined their dynamic expression 4–12 weeks post-infection. With the extension of *E. multilocularis* infection time, the levels of mmu-miR-103-3p, mmu-miR-107-3p, mmu-miR-21a-3p and mmu-miR-146a-5p were significantly upregulated compared with the early stage of infection (4-week post-infection) (Fig. 3). The expression of mmu-miR-339-5p was significantly upregulated at the early stage of infection, but its expression showed no difference after 4-week infection (Fig. 3). Conversely, mmu-miR-222-3p was significantly downregulated in the course of *E. multilocularis* infection (Fig. 3 and online Supplementary Table S6).

GO enrichment of differentially expressed miRNAs targets

Using the softwares TargetScan (Agarwal *et al.* 2015), miRanda (Betel *et al.* 2008) and RNAhybrid (Lai and Meyer, 2016) to predict transcript targets from the mouse genome for the identified miRNAs, 16 115 predicted targets were obtained for 58 differentially expressed miRNAs. According to *P* value of the enrichment analysis of target genes, top 51 enriched GO terms of biological process, molecular function and cellular component were shown in Fig. 4, respectively. The notable GO terms were the immune response, signal transduction, gene expression regulation and metabolism.

Seven E. multilocularis*-derived circulating miRNAs were identified in the serum*

To determine whether *E. multilocularis*-derived circulating miRNAs are present in the infected serum, we carried out a comparative analysis between the infected library and the control library. After removal of sequences mapping to the mouse genomes and the overlapped sequences in both libraries, only 0·43% sequences were mapped to the *E. multilocularis* genome. By analysis of reads that mapped to the *E. multilocularis* genome using the miRDeep program, seven known *E. multilocularis* miRNAs, including emu-miR-10, emu-let-7, emu-

https://doi.org/10.1017/S0031182017000300 Published online by Cambridge University Press

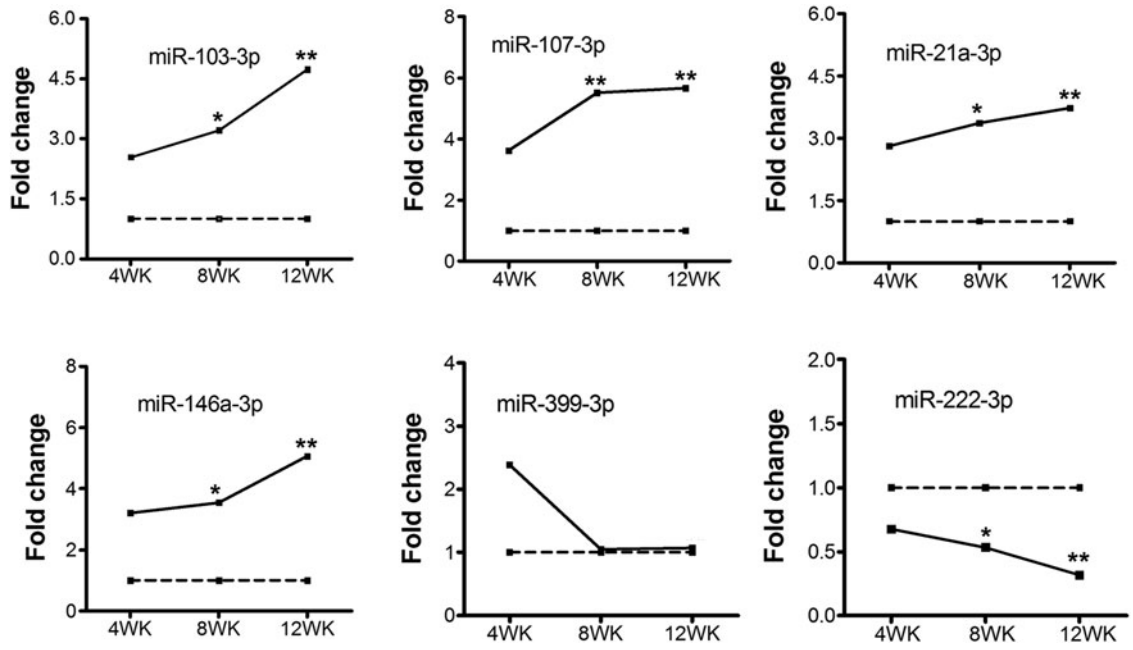

Fig. 3. Dynamic expression of host-origin miRNAs in the sera of mice infected with *Echinococcus multilocularis* 4–12 weeks post-infection. miRNA expression was quantified by qRT–PCR and normalized to cel-miR-39, and fold changes were calculated as the ratio of values from infected *vs* uninfected mice. The relative miRNA expression in each uninfected sera was set as 1·0. Data were expressed as means ± S.D. (*$P < 0{\cdot}05$, **$P < 0{\cdot}01$). Data for the final analysis were from three independent experiments.

miR-71, emu-miR-2b, emu-miR-4989, emu-miR-2c-3p and emu-miR-277, were identified from the *E. multilocularis*-infected serum (Table 2). To further confirm the presence of these miRNAs in the infected sera, seven miRNAs were assessed by a stem-loop-based semi-qPCR. Both RT–PCR and sequencing results showed that all seven miRNAs were detected in the sera of *E. multilocularis*-infected mice (Fig. 5A). Both emu-miR-10 and emu-miR-227 were specifically amplified in all the sera from mice infected with *E. multilocularis*, but the rest were likely to be nonspecifically amplified in the control probably due to the high sequence identity with mouse miRNAs (Fig. 5A and online Supplementary Table S1). The incorporation of LNA™ in primers can increase the specificity and sensitivity of PCR. Hence, we examined the expression of three parasite-derived miRNAs (emu-miR-10, emu-miR-227 and emu-miR-71) by qPCR using LNA-modified primers (online Supplementary Table S1). Three parasite miRNAs displayed a significant signal level in the serum of *E. multilocularis*-infected mice compared with the control group (Fig. 5B and online Supplementary Table S7).

DISCUSSION

Increasing evidence shows that tissue-specific and circulating miRNA in mammalian hosts are dysregulated during an active helminthic infection, hinting their regulatory roles in parasite–host interplay. It was reported that the expression of miRNAs in the sheep intestinal tissue was altered by *Echinococcus granulosus* infection (Jiang *et al.* 2016). Recently, our study also found that *E. multilocularis* infection disturbed the small RNA-induced silencing pathway via dysregulation of miRNAs (Jin *et al.* 2017). To date, no studies have examined the profile of circulating miRNAs in hosts during *E. multilocularis* infection. In this study, we found that 58 host-circulating miRNAs were dysregulated in the serum of *E. multilocularis*-infected mouse. The expression levels of six selected differentially expressed miRNAs were consistent with the sequencing data. We further assessed the dynamic expression of six validated miRNAs in the sera from mice with *E. multilocularis* infection. Besides mmu-miR-339-5p, the expression levels of four upregulated miRNAs were elevated gradually and the mmu-miR-222-3p (one of downregulated miRNAs) was decreased gradually with the extension of *E. multilocularis* infection time. To understand the regulatory roles of differentially expressed miRNAs, the target genes were predicted. Among these target genes, some are involved in host immune responses. For example, it is worth mentioning that miRNA-146a as a negative regulator is involved in innate immunity and inflammatory response by targeting IRAK1 (interleukin-1 receptor-associated kinase 1) or TRAF6 [tumour necrosis factor (TNF) receptor-associated factor 6) (Taganov *et al.* 2006; Saba *et al.* 2014). Its expression was shown to be induced after *Plasmodium, Cryptosporidium* and *Toxoplasma* infections (Cannella *et al.* 2014; Judice

https://doi.org/10.1017/S0031182017000300 Published online by Cambridge University Press

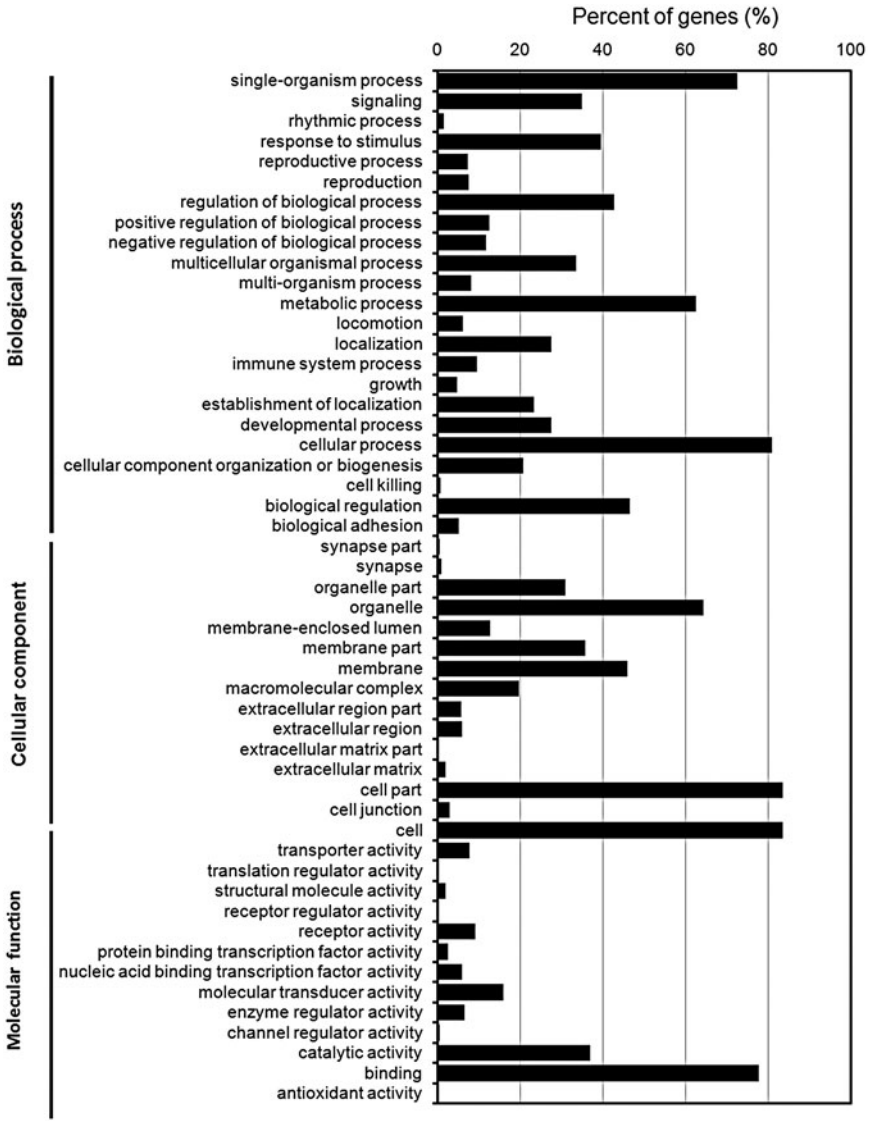

Fig. 4. GO molecular function annotations of the target genes of differentially expressed miRNAs. According to *P* value, top GO terms of biological process, molecular function and cellular component were shown, respectively.

Table 2. Seven parasite-derived circulating miRNAs in the *Echinococcus multilocularis*-infected mouse sera

miRNA	Mature sequence (5′–3′)	Reads
emu-miR-10	CACCCUGUAGACCCGAGUUUGA	926
emu-let-7	UGAGGUAGUGUUUCGAAUGUCU	83
emu-miR-71	UGAAAGACGAUGGUAGUGAGAUA	4
emu-miR-2b	UAUCACAGCCCUGCUUGGGACAC	2
emu-miR-4989	AAAAUGCACCAACUAUCUGAGA	1
emu-miR-2c-3p	UCACAGCCAAUAUUGAUGAAC	1
emu-miR-277	UAAAUGCAUUUUCUGGCCCGUAA	1

et al. 2016). We also found that the mmu-miR-146a was predominantly upregulated in the mouse sera after *E. multilocularis* infection. The mmu-miR-103/107, whose expression was also upregulated in the *E. multilocularis*-infected mouse sera, were predicted to target a negative regulator of the toll-like receptor signalling, TRAF3 (TNF receptor-associated factor 3).

Circulating parasite-derived miRNAs are existed stably in blood plasma or sera of humans or animals during parasite infection. The significance of serum miRNAs as biochemical markers for helminth infections has been gradually recognized (Mitchell *et al*. 2008; Hoy *et al*. 2014). To the best of our knowledge, this is the first report of circulating parasite-derived miRNAs in the serum from

https://doi.org/10.1017/S0031182017000300 Published online by Cambridge University Press

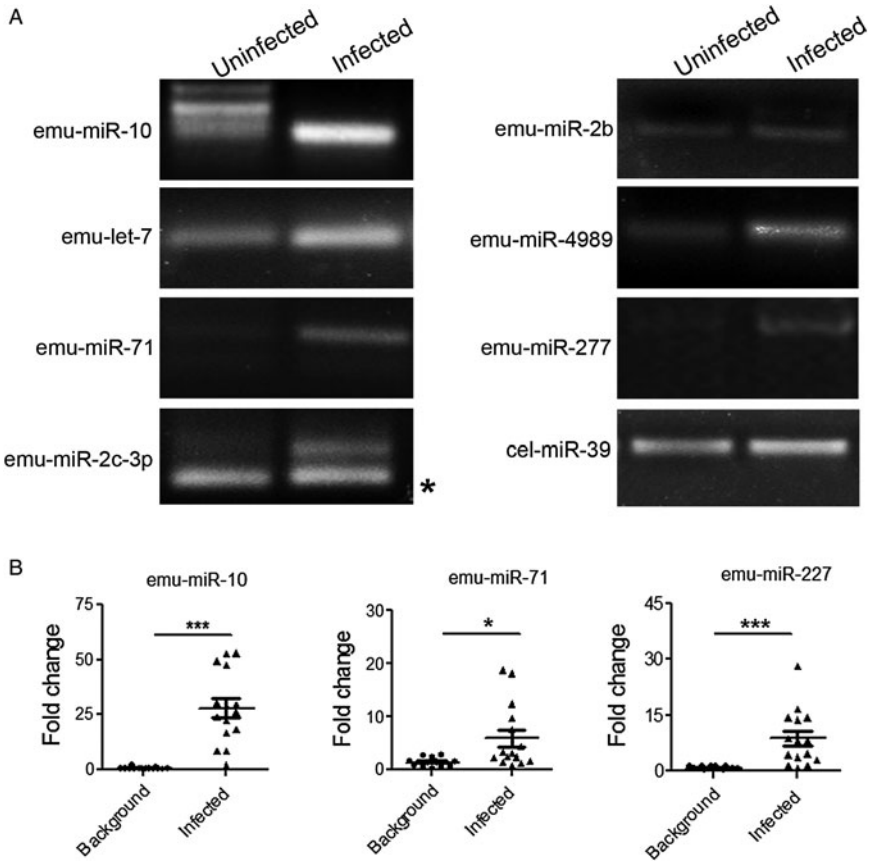

Fig. 5. Detection of parasite-derived miRNAs in mouse sera during *Echinococcus multilocularis* infection. (A) Semi-quantitative PCR analysis of *E. multilocularis*-specific miRNAs identified in the infected and uninfected sera; (B) Detection of parasite-derived miRNAs in mouse sera during *E. multilocularis* infections. miRNAs were quantified by qRT–PCR and normalized to cel-miR-39, and fold changes were calculated as the ratio of normalized values in infected sera compared with the background in uninfected sera. A total of 15 sera samples in each group were tested and each symbol represents data from one individual mouse (*$P < 0{\cdot}05$, *** $P < 0{\cdot}001$).

E. multilocularis-infected mouse. Seven *E. multilocularis*-derived miRNAs were identified in the infected mouse serum, indicating the secretion of miRNAs by parasites during infection. emu-miR-10 was one of the most highly abundant in *E. multilocularis*-infected serum. Similarly, sja-miR-10 was also detected in the plasma of rabbits infected with *S. japonicum* (Cheng *et al*. 2013), providing an independent validation for the presence of parasite-derived miRNAs in the serum. Parasite-origin miR-71 was detected in host serum, plasma, body fluids and microenvironments that surround the parasites (Buck *et al*. 2014; Hoy *et al*. 2014; Quintana *et al*. 2015). miR-71 was already found to be packed into exosomes or exosome-like extracellular vesicles from a trematode (Bernal *et al*. 2014, 2015; Fromm *et al*. 2016; Zhu *et al*. 2016) and nematodes (Buck *et al*. 2014; Zamanian *et al*. 2015). Our recent result showed that emu-miR-71 had a capacity of modulation of NO production by macrophages, suggesting a role in parasite–host interactions (Zheng *et al*. 2016). Similarly, sja-miR-227 was identified in the sera of *Schistoma mansoni*-infected mice and patient (Hoy *et al*. 2014; Cai *et al*. 2015). The extensive presence of *E. multilocularis*-specific miRNAs in host serum may suggest that *E. multilocularis* releases miRNAs into host serum via exosome-like EVs (Zheng *et al*. 2017). The discovery of parasite-specific miRNA profiles in the host serum has raised great interest in their potential as diagnostic and prognostic biomarkers and in the biological functions during the infection of *Echinococcus* species.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0031182017000300.

ACKNOWLEDGEMENTS

The author would like to thank Miss Jing yang and Meng Su for help in serum collection and also thank the reviewer and editor for their constructive suggestions.

FINANCIAL SUPPORT

The study was financially supported by grants from the National Key Basic Research Program (973 program) of

China (No. 2015CB150300), the National Natural Science Foundation of China (Nos 31201900 and 31472185) and State Public-interest Institution Basal Research Fund (Nos 1610312016017 and 1610312017017). The funders had no role in study design, data collection and analysis, decision to publish and preparation of the manuscript.

REFERENCES

Agarwal, V., Bell, G. W., Nam, J.-W. and Bartel, D. P. (2015). Predicting effective microRNA target sites in mammalian mRNAs. *eLife* **4**, e05005.

Baltimore, D., Boldin, M. P., O'Connell, R. M., Rao, D. S. and Taganov, K. D. (2008). MicroRNAs: new regulators of immune cell development and function. *Nature Immunology* **9**, 839–845.

Bernal, D., Trelis, M., Montaner, S., Cantalapiedra, F., Galiano, A., Hackenberg, M. and Marcilla, A. (2014). Surface analysis of *Dicrocoelium dendriticum*. The molecular characterization of exosomes reveals the presence of miRNAs. *Journal of Proteomics* **105**, 232–241.

Betel, D., Wilson, M., Gabow, A., Marks, D. S. and Sander, C. (2008). The microRNA.org resource: targets and expression. *Nucleic Acids Research* **36**, D149–D153.

Buck, A. H., Coakley, G., Simbari, F., McSorley, H. J., Quintana, J. F., Le Bihan, T., Kumar, S., Abreu-Goodger, C., Lear, M., Harcus, Y., Ceroni, A., Babayan, S. A., Blaxter, M., Ivens, A. and Maizels, R. M. (2014). Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. *Nature Communications* **5**, 5488.

Cai, P., Gobert, G. N., You, H., Duke, M. and McManus, D. P. (2015). Circulating miRNAs: potential novel biomarkers for hepatopathology progression and diagnosis of *Schistosomiasis Japonica* in two murine models. *PLoS Neglected Tropical Diseases* **9**, e0003965.

Cai, P., Gobert, G. N. and McManus, D. P. (2016). MicroRNAs in parasitic helminthiases: current status and future perspectives. *Trends in Parasitology* **32**, 71–86.

Cannella, D., Brenier-Pinchart, M. P., Braun, L., van Rooyen, J. M., Bougdour, A., Bastien, O., Behnke, M. S., Curt, R. L., Curt, A., Saeij, J. P., Sibley, L. D., Pelloux, H. and Hakimi, M. A. (2014). miR-146a and miR-155 delineate a MicroRNA fingerprint associated with *Toxoplasma* persistence in the host brain. *Cell Reports* **6**, 928–937.

Cheng, G., Luo, R., Hu, C., Cao, J. and Jin, Y. (2013). Deep sequencing-based identification of pathogen-specific microRNAs in the plasma of rabbits infected with *Schistosoma japonicum*. *Parasitology* **140**, 1751–1761.

Craig, P. S., McManus, D. P., Lightowlers, M. W., Chabalgoity, J. A., Garcia, H. H., Gavidia, C. M., Gilman, R. H., Gonzalez, A. E., Lorca, M., Naquira, C., Nieto, A. and Schantz, P. M. (2007). Prevention and control of cystic echinococcosis. *The Lancet Infectious Diseases* **7**, 385–394.

Cucher, M., Prada, L., Mourglia-Ettlin, G., Dematteis, S., Camicia, F., Asurmendi, S. and Rosenzvit, M. (2011). Identification of *Echinococcus granulosus* microRNAs and their expression in different life cycle stages and parasite genotypes. *International Journal for Parasitology* **41**, 439–448.

Cucher, M., Macchiaroli, N., Kamenetzky, L., Maldonado, L., Brehm, K. and Rosenzvit, M. C. (2015). High-throughput characterization of *Echinococcus* spp. metacestode miRNomes. *International Journal for Parasitology* **45**, 253–267.

Czermak, B. V., Akhan, O., Hiemetzberger, R., Zelger, B., Vogel, W., Jaschke, W., Rieger, M., Kim, S. Y. and Lim, J. H. (2008). Echinococcosis of the liver. *Abdominal Imaging* **33**, 133–143.

Dennis, G., Sherman, B. T., Hosack, D. A., Yang, J., Gao, W., Lane, H. C. and Lempicki, R. A. (2003). DAVID: database for annotation, visualization, and integrated discovery. *Genome Biology* **4**, R60.

Deplazes, P. and Eckert, J. (2001). Veterinary aspects of alveolar echinococcosis – a zoonosis of public health significance. *Veterinary Parasitology* **98**, 65–87.

Fromm, B., Trelis, M., Hackenberg, M., Cantalapiedra, F., Bernal, D. and Marcilla, A. (2015). The revised microRNA complement of *Fasciola hepatica* reveals a plethora of overlooked microRNAs and evidence for enrichment of immuno-regulatory microRNAs in extracellular vesicles. *International Journal for Parasitology* **45**, 697–702.

Fromm, B., Ovchinnikov, V., Hoye, E., Bernal, D., Hackenberg, M. and Marcilla, A. (2016). On the presence and immunoregulatory functions of extracellular microRNAs in the trematode *Fasciola hepatica*. *Parasite Immunology*. doi: 10.1111/pim.12399.

He, X., Sai, X., Chen, C., Zhang, Y., Xu, X., Zhang, D. and Pan, W. (2013). Host serum miR-223 is a potential new biomarker for *Schistosoma japonicum* infection and the response to chemotherapy. *Parasites & Vectors* **6**, 272.

Hoy, A. M., Lundie, R. J., Ivens, A., Quintana, J. F., Nausch, N., Forster, T., Jones, F., Kabatereine, N. B., Dunne, D. W. and Mutapi, F. (2014). Parasite-derived microRNAs in host serum as novel biomarkers of helminth infection. *PLoS Neglected Tropical Diseases* **8**, e2701.

Jia, B., Chang, Z., Wei, X., Lu, H., Yin, J., Jiang, N. and Chen, Q. (2014). Plasma microRNAs are promising novel biomarkers for the early detection of *Toxoplasma gondii* infection. *Parasites & Vectors* **7**, 433.

Jiang, S., Li, X., Wang, X., Ban, Q., Hui, W. and Jia, B. (2016). MicroRNA profiling of the intestinal tissue of Kazakh sheep after experimental *Echinococcus granulosus* infection, using a high-throughput approach. *Parasite* **23**, 23.

Jin, X., Guo, X., Zhu, D., Ayaz, M. and Zheng, Y. (2017). miRNA profiling in the mice in response to *Echinococcus multilocularis* infection. *Acta Tropica* **166**, 39–44.

Judice, C. C., Bourgard, C., Kayano, A. C., Albrecht, L. and Costa, F. T. (2016). MicroRNAs in the host-apicomplexan parasites interactions: a review of immunopathological aspects. *Frontiers in Cellular and Infection Microbiology* **6**, 5.

Lai, D. and Meyer, I. M. (2016). A comprehensive comparison of general RNA–RNA interaction prediction methods. *Nucleic Acids Research* **44**, e61.

Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L., Peterson, A., Noteboom, J., O'Briant, K. C. and Allen, A. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 10513–10518.

Quintana, J. F., Makepeace, B. L., Babayan, S. A., Ivens, A., Pfarr, K. M., Blaxter, M., Debrah, A., Wanji, S., Ngangyung, H. F., Bah, G. S., Tanya, V. N., Taylor, D. W., Hoerauf, A. and Buck, A. H. (2015). Extracellular Onchocerca-derived small RNAs in host nodules and blood. *Parasites & Vectors* **8**, 58.

Saba, R., Sorensen, D. L. and Booth, S. A. (2014). MicroRNA-146a: a dominant, negative regulator of the innate immune response. *Frontiers in Immunology* **5**, 578.

Salone, V. and Rederstorff, M. (2015). Stem-loop RT–PCR based quantification of small non-coding RNAs. *Methods in Molecular Biology* **1296**, 103–108.

Silakit, R., Loilome, W., Yongvanit, P., Chusorn, P., Techasen, A., Boonmars, T., Khuntikeo, N., Chamadol, N., Pairojkul, C. and Namwat, N. (2014). Circulating miR-192 in liver fluke-associated cholangiocarcinoma patients: a prospective prognostic indicator. *Journal of Hepatobiliary Pancreatic Science* **21**, 864–872.

Sonkoly, E. and Pivarcsi, A. (2009). Advances in microRNAs: implications for immunity and inflammatory diseases. *Journal of Cellular and Molecular Medicine* **13**, 24–38.

Taganov, K. D., Boldin, M. P., Chang, K. J. and Baltimore, D. (2006). NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 12481–12486.

Torgerson, P. R., Keller, K., Magnotta, M. and Ragland, N. (2010). The global burden of alveolar echinococcosis. *PLoS Neglected Tropical Diseases* **4**, e722.

Wang, J.-F., Yu, M.-L., Yu, G., Bian, J.-J., Deng, X.-M., Wan, X.-J. and Zhu, K.-M. (2010). Serum miR-146a and miR-223 as potential new biomarkers for sepsis. *Biochemical and Biophysical Research Communications* **394**, 184–188.

Wang, J.-Y., Gao, C.-H., Steverding, D., Wang, X., Shi, F. and Yang, Y.-T. (2013). Differential diagnosis of cystic and alveolar echinococcosis using an immunochromatographic test based on the detection of specific antibodies. *Parasitology Research* **112**, 3627–3633.

Xu, M. J., Zhou, D. H., Nisbet, A. J., Huang, S. Y., Fan, Y. F. and Zhu, X. Q. (2013). Characterization of mouse brain microRNAs after infection with cyst-forming *Toxoplasma gondii*. *Parasites & Vectors* **6**, 154.

Yamano, K., Miyoshi, M., Goto, A. and Kawase, S. (2014). Time course of the antibody response in humans compared with rats experimentally infected with hepatic alveolar echinococcosis. *Journal of Helminthology* **88**, 24–31.

Zamanian, M., Fraser, L. M., Agbedanu, P. N., Harischandra, H., Moorhead, A. R., Day, T. A., Bartholomay, L. C. and Kimber, M. J. (2015). Release of small RNA-containing exosome-like vesicles from the human filarial parasite brugia malayi. *PLoS Neglected Tropical Diseases* **9**, e0004069.

Zhang, C., Wang, J., Lu, G., Li, J., Lu, X., Mantion, G., Vuitton, D. A., Wen, H. and Lin, R. (2012). Hepatocyte proliferation/growth arrest balance in the liver of mice during *E. multilocularis* infection: a coordinated 3-stage course. *PLoS ONE* **7**, e30127.

Zhang, W., Zhang, Z., Wu, W., Shi, B., Li, J., Zhou, X., Wen, H. and McManus, D. P. (2015). Epidemiology and control of echinococcosis in

central Asia, with particular reference to the People's Republic of China. *Acta Tropica* **141**, 235–243.

Zheng, Y., Cai, X. and Bradley, J. E. (2013). microRNAs in parasites and parasite infection. *RNA Biology* **10**, 371–379.

Zheng, Y., Guo, X., He, W., Shao, Z., Zhang, X., Yang, J., Shen, Y., Luo, X. and Cao, J. (2016). Effects of *Echinococcus multilocularis* miR-71 mimics on murine macrophage RAW264.7 cells. *International Immunopharmacology* **34**, 259–262.

Zheng, Y., Guo, X., Su, M., Guo, A., Ding, J., Yang, J., Xiang, H., Cao, X., Zhang, S., Ayaz, M. and Luo, X. (2017). Regulatory effects of *Echinococcus multilocularis* extracellular vesicles on RAW264.7 macrophages. *Veterinary Parasitology* **235**, 29–36.

Zhu, L., Liu, J., Dao, J., Lu, K., Li, H., Gu, H., Liu, J., Feng, X. and Cheng, G. (2016). Molecular characterization of *S. japonicum* exosome-like vesicles reveals their regulatory roles in parasite–host interactions. *Scientific Reports* **6**, 25885.

https://doi.org/10.1017/S0031182017000300 Published online by Cambridge University Press