MicroRNA expression profile of HCT-8 cells in the early phase of Cryptosporidium parvum infection

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Abstract

Background: Cryptosporidium parvum is an important zoonotic parasitic disease worldwide, but the molecular mechanisms of the host–parasite interaction are not fully understood. Noncoding microRNAs (miRNAs) are considered key regulators of parasitic diseases. Therefore, we used microarray, qPCR, and bioinformatic analyses to investigate the intestinal epithelial miRNA expression profile after Cryptosporidium parvum infection.

Results: Twenty miRNAs were differentially expressed after infection (four upregulated and 16 downregulated). Further analysis of the differentially expressed miRNAs revealed that many important cellular responses were triggered by Cryptosporidium parvum infection, including cell apoptosis and the inflammatory and immune responses.

Conclusions: This study demonstrates for the first time that the miRNA expression profile of human intestinal epithelium cells is altered by C. parvum infection. This dysregulation of miRNA expression may contribute to the regulation of host biological processes in response to C. parvum infection, including cell apoptosis and the immune responses. These results provide new insight into the regulatory mechanisms of host miRNAs during cryptosporidiosis, which may offer potential targets for future C. parvum control strategies.

Keywords: Cryptosporidium parvum, Intestinal epithelium, Infection, MicroRNA

Background

Cryptosporidium is a genus of protozoan parasites that infect the gastrointestinal epithelium and other mucosal surfaces of their hosts, which include humans and domestic and wild animals worldwide [1–3]. Cryptosporidium is a major cause of moderate to severe diarrhea in children younger than 2 years, particularly infants, and is second only to rotavirus in this regard [4]. Morbidity and mortality are associated with cryptosporidial infections in patients with acquired immunodeficiency syndrome (AIDS) and young children [1, 5]. Cryptosporidium parvum is one of the species most commonly involved in human cryptosporidial infections [1]. Despite intensive efforts over the past 30 years, the precise molecular pathogenic mechanisms of cryptosporidial infection are not fully understood.

MicroRNAs (miRNAs) are endogenous noncoding small RNAs (22 nucleotides) that regulate gene expression at the posttranscriptional level and play important roles in the regulation of diverse pathological processes, including cell proliferation, differentiation, metabolism, the immune response, and apoptosis [6–8]. The miRNAs in mammalian cells have been shown to play crucial roles in the cellular responses to infection by diverse pathogens, including viruses, parasites, and bacteria [9–11]. Increasing evidence suggests that host miRNAs help the host to clear parasites by regulating the toll-like receptor 4 (TLR4) signaling pathways and the release of antimicrobial peptides [12, 13]. For example, C. parvum infection reduces the expression of the let-7 family miRNAs in biliary epithelial cells, which increases SNAP23 expression, coordinating the release of exosomes carrying antimicrobial-peptide in response to C. parvum infection [13]. The upregulation of
miR-27b expression enhances the anti-C. parvum responses of biliary epithelial cells [14]. Some evidence suggests that host miRNAs are also used by C. parvum to enhance its own survival. For example, suppressor of cytokine signaling (SOCS) proteins are negative regulators of cytokine signaling [15]. Cryptosporidium parvum infection downregulates the expression of miR-98 and let-7 to induce SOCS protein expression in biliary epithelial cells [16]. The upregulation of miR-21 after C. parvum infection inhibits the activation of TLR4/NF-κB signaling in biliary epithelial cells by targeting PDCD4, a proinflammatory protein that promotes the activation of NF-κB [17]. Therefore, studying the changes in the host miRNA expression profile after C. parvum infection will extend our understanding of the interaction between C. parvum and its host.

There has been no report of the changes in the miRNA expression profile in the intestinal epithelium after C. parvum infection. Therefore, the objective of this study was to analyze the intestinal epithelial miRNA expression profile after C. parvum infection using microarray and bioinformatics analyses in an in vitro model using human ileocecal HCT-8 adenocarcinoma cells.

Methods

Cell and parasite

Human ileocecal adenocarcinoma (HCT-8) cells (American Type Culture Collection, Manassas, VA) were cultured and maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 4 mmol/L L-glutamine, 100 U/mL penicillin, and 100 U/mL streptomycin at 37°C in a humidified 5% CO₂ incubator.

Neonatal calves were purchased from Ruiya Animal Husbandry Co., Ltd. (Zhengzhou, China). Oocysts of C. parvum (IId subtype) were maintained in infected neonatal calves, purified from their feces, and stored at 4°C until use. Before use, the oocysts were washed with phosphate-buffered saline (PBS), treated with 10% hypochlorite for 10 min at 4°C, and washed again [18]. The infected calf was killed by anesthesia (113 mg sodium pentobarbital/kg, Intravenous injection) and carcass was treated harmlessly by a specialized agency of our college.

The infection assays have been reported previously [18]. Briefly, cell monolayers in six-well cell culture dishes were inoculated with 100 μL of purified oocysts (1 × 10⁹/well) in DMEM, that the oocyst:host cell ratio is 5:1. Heat-inactivated (100°C, 5 min) oocysts were used as the controls. After 2.5 h, oocysts and sporozoites remaining in the supernatant were removed and replaced with DMEM containing 2% fetal bovine serum, 4 mmol/L L-glutamine, 100 U/mL penicillin, 100 g/mL streptomycin, 0.3 g/L bicarbonate, and 0.02 g/L taurocholate. The plates were incubated at 37°C in a humidified 5% CO₂ incubator.

Total RNA isolation and quality control

At 4 and 12 h postinfection, the cells were washed three times with PBS, and 1 mL of TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) was added to each well. The total RNA from three uninfected separate monolayers and three infected separate monolayers were isolated according to the manufacturer’s protocol.

The quality of the RNA was measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). RNA purity was assessed from the absorbance (A) of the solutions at various wavelengths: 2.0 > A₂₆₀/A₂₈₀ > 1.8 and A₂₆₀/A₂₃₀ > 2.0 were considered to indicate high purity. RNA integrity was determined from the 28S:18S rRNA ratio determined with an electrophoretic analysis.

Microarray analysis

miRNA microarrays based on miRBase v20.0 (http://www.mirbase.org/) were used to study the expression profiles of 2555 mature miRNAs. Microarray chips are synthesized by LC Sciences and have patent rights. The chips are made by a flexible, in situ synthesis method using conventional chemistry. The technology encompasses a novel high-throughput biopolymer synthesis chemistry carried out using a new class of microfluidic reaction devices and an advanced digital light synthesizer apparatus and is developed for carrying out picoliter scale chemical and/or biochemical reactions. Briefly, Hybridization was performed using the µParaflo® microfluidic array technology (LC Sciences, Hangzhou, China), according to the manual. The assay started from 4 to 8 μg total RNA sample were 3′-extended with a poly(A) tail using poly(A) polymerase. An oligonucleotide tag that binding to Cy3 dyes in later chip hybridization was then ligated to the poly(A) tail. Hybridization was performed overnight on a µParaflo microfluidic chip using a micro-circulation pump (Atactic Technologies). On the microfluidic chip, each detection probe consisted of a chemically modified nucleotide coding segment complementary to target microRNA (from miRBase, http://www.mirbase.org/). The detection probes were made by in situ synthesis using PGR (photogenerated reagent) chemistry. The hybridization melting temperatures were balanced by chemical modifications of the detection probes. Hybridization used 100 μL 6xSSPE buffer (0.90 M NaCl, 60 mM Na₂HPO₄, 6 mM EDTA, pH 6.8) containing 25% formamide at 34°C. After RNA hybridization, oligonucleotide tag-conjugating Cy3 dye were circulated through the microfluidic chip for dye staining. Fluorescence images were collected using a laser scanner.
Validation of microarray data with quantitative PCR (qPCR) analysis

miRNAs were prepared with an All-in-One™ miRNA qRT–PCR Detection Kit (GeneCopoeia, Rockville, MD, USA), according to the manufacturer’s instructions. Briefly, the extracted RNA was reverse-transcribed in the presence of a poly(A) polymerase and an oligo-dT adaptor. Quantitative PCR was then performed with SYBR Green detection using a forward primer specific for the mature miRNA sequence and a universal adaptor reverse primer. The forward primers used for the qPCRs are shown in Table 1. Each RNA was run in triplicate wells with three biological replicates, including three uninfected separate monolayers and three infected separate monolayers. The cycling protocol was: 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 20 s, with a final extension at 72 °C for 20 s. When all cycles were finished, a melting-curve analysis was performed. The baseline and cycle threshold (Ct) values were automatically determined for all plates with the Roche LightCycler® 96 Real-Time PCR System. The miRNA expression levels in each sample were normalized to the housekeeping gene U6.

Results

Differentially expressed miRNAs

A total of 2555 mature human miRNA sequences were detected in C. parvum-infected and uninfected HCT-8 cells using miRNA microarrays (Sanger miRBase v20.0). A dendrogram produced with a hierarchical clustering analysis of the miRNAs differentially expressed in C. parvum-infected and -uninfected cells is shown in Fig. 1. In total, 20 miRNAs (four upregulated and 16 downregulated) were significantly differentially expressed in the infected HCT-8 cells compared to uninfected cells. These differentially expressed miRNAs (with fold changes ≥2 and P ≤ 0.05) are shown in Table 2 with P values. From this table, it is clear that downregulated miRNAs were more frequent than upregulated miRNAs (P < 0.05). Among these miRNAs, hsa-miR-942-5p was most strongly upregulated and hsa-miR-4689 was most strongly downregulated.

Validation of miRNA microarray data with qPCR

Six selected miRNAs and the housekeeping U6 mRNA were assayed with qPCR to confirm the expression
profiles identified with the microarray analysis. The expression patterns determined with qPCR agreed well with those determined from the microarray data (Fig. 2).

**Predicting the target genes of differentially expressed miRNAs**

We predicted the target genes for all the miRNAs differentially expressed in HCT-8 cells after *C. parvum* infection. Target genes were predicted for only 18 of the 20 differentially expressed miRNAs using two different online software programs, TargetScan and miRDB.

**GO analysis of differentially expressed miRNAs**

To further understand the functions of the differentially expressed miRNAs, a GO analysis of the target genes was performed. DAVID gene annotation was applied to explain the biological effects of the differentially expressed miRNAs based on their target genes. The GO annotations of differentially expressed miRNAs (with $P < 0.05$) are shown in Fig. 3. The GO-specific functions mainly involved biological processes (e.g., transcription initiation from the RNA polymerase II promoter, apoptotic process, and immune response), cell components (e.g., membrane, actin cytoskeleton, focal adhesion, plasma membrane, cell surface, and apical part of the cell), and molecular functions (e.g., protein binding, ATP binding, calcium ion binding). Among the GO annotations (with $P < 0.05$), hsa-miR-34b-5p, hsa-miR-942-5p, hsa-miR-3591-3p, hsa-miR-18b-3p and hsa-miR-3976 were involved in the regulation of apoptotic or autophagy processes, hsa-miR-34b-5p and hsa-miR-3591-3p were involved in the regulation of the immune response (Additional file 1: Figure S1). Thus, the GO analysis of

### Table 2 Differentially expressed miRNAs in the infected HCT-8 cells compared to uninfected cells

| Infection time | hsa-microRNAs | Log2 fold change | $P$-value |
|----------------|---------------|------------------|-----------|
| 4 h            | hsa-miR-122-5p| +4.85            | 0.01      |
|                | hsa-miR-3591-3p| −12.29          | 0.02      |
|                | hsa-miR-6074 | −11.12           | 0.03      |
|                | hsa-miR-454-5p| −12.16           | 0.04      |
|                | hsa-miR-34b-5p| −12.19           | 0.04      |
|                | hsa-miR-4685-3p| −7.02           | 0.03      |
|                | hsa-miR-1908-3p| −9.88           | 0.04      |
|                | hsa-miR-942-5p| +15.26           | 0.05      |
|                | hsa-miR-5580-3p| +3.42           | 0.03      |
|                | hsa-miR-6763-5p| +2.99           | 0.03      |
|                | hsa-miR-1813p-3p| −8.99          | 0.009     |
|                | hsa-miR-18b-3p| −10.94          | 0.01      |
|                | hsa-miR-4689 | −12.30           | 0.02      |
|                | hsa-miR-1256 | −7.35            | 0.02      |
|                | hsa-miR-3976 | −10.42           | 0.03      |
|                | hsa-miR-1287-3p| −6.62           | 0.05      |

* + represents up-regulation, − represents down-regulation
the differentially expressed miRNA target genes revealed that some have potentially important biological functions in regulating the host against *C. parvum* infection.

**KEGG pathway analysis of differentially expressed miRNAs**
The main KEGG pathway annotations of the target genes (with P < 0.05) are shown in Table 3. Among these KEGG pathways, several were associated with the immune response (e.g., cytokine receptor interaction, chemokine signaling pathway, and inflammatory mediator regulation of TRP channels), apoptosis (e.g., MAPK signaling pathway and ERBB signaling pathway), cytoskeleton (e.g., actin cytoskeleton and WNT signaling pathway), and cell adhesion (e.g., cell adhesion molecules).

**Regulatory network of miRNAs and target genes**
To investigate the interactions between the miRNAs and their target genes, we performed an miRNA–target gene network analysis (Fig. 4). From the network of the miRNA and their target genes at 4 h after *C. parvum* infection (Fig. 4a), we found among seven differentially expressed miRNAs at 4 h hsa-miR-34b-5p and hsa-miR-3591-3p have a connection with six differentially expressed miRNAs. Among eleven differentially expressed miRNAs at 12 h, hsa-miR-942-5p, hsa-let-7b-3p and hsa-miR-6721-5p have a connection with ten differentially expressed miRNAs (Fig. 4b). According to these datasets, the key miRNAs among the differentially expressed miRNAs were hsa-miR-34b-5p, hsa-miR-3591-3p, hsa-miR-942-5p, hsa-let-7b-3p, and hsa-miR-6721-5p. Their common target genes of the key miRNAs at 12 h were shown in Fig. 4c.

**Discussion**
Increasing evidence suggests that miRNAs regulate gene expression at the posttranscriptional level and thus affect a variety of biological processes, including the pathogenesis and progression of various diseases [30]. miRNAs also play important roles in the regulation of complex parasite–host interactions [10, 12, 14, 15]. *Cryptosporidium parvum*-induced miRNA expression in human biliary epithelial cells has already been investigated and analyzed [10]. However, there has been no report of the miRNA expression profile of human intestinal epithelial cells infected with *C. parvum*. Therefore, we analyzed the changes in the miRNA profile of HCT-8 cells at 4 h and 12 h after *C. parvum* infection using microarray technology. Our results show that most miRNAs were not significantly differentially expressed in the infected HCT-8 cells compared to uninfected cells. However, some differentially expressed miRNAs were detected (four upregulated and 16 downregulated), implying that *C. parvum* infection alters the miRNA profile of intestinal epithelial cells. However, the differentially expressed miRNAs in the infected HCT-8 cells differed from those in biliary epithelial cells [10]. The changes in these specific miRNAs induced by *C. parvum* might be involved in the regulation of human intestinal epithelial cells in response to *C. parvum* infection. This is the first report to describe the alterations in miRNA expression induced by *C. parvum* infection in human intestinal epithelial cells, and extends our understanding of the interplay between *C. parvum* and its host at the miRNA level. The identification of the functions of these miRNA sheds new light on the molecular mechanisms triggered by *C. parvum* infection. Therefore, in this study, GO and KEGG analyses were used to identify the functions of the predicted target genes involved in *C. parvum* infection. Cell apoptosis is one of the ancient antiparasite strategies used by a host after its invasion by a parasite [31–34]. Research has shown that the early phase of *C. parvum* infection results in the apoptosis of HCT-8 cells [35]. However, miRNA-mediated cell apoptosis after *C.
parvum infection has not been reported. Our results suggest that the functions of hsa-miR-34b-5p, hsa-miR-942-5p, hsa-miR-18b-3p, hsa-miR-3976 and hsa-miR-3591-3p, most of which (not hsa-miR-942-5p) were downregulated after C. parvum infection, regulate apoptotic processes. Several reports have shown that hsa-miR-34b, hsa-miR-18a, and hsa-miR-942 regulate cell apoptosis in response to microbial infection. For example, upregulated hsa-miR-18a in macrophages after Toxoplasma gondii infection leads to the survival of the infected macrophages by targeting BIM [36]. Upregulated hsa-miR-30c-1 in human macrophages is involved in an anti-apoptosis response to T. gondii infection [37]. The downregulation of hsa-miR-34a induces cell apoptosis in Influenza A virus-infected A549 cells [38], and the downregulation of hsa-miR-942 enhances the apoptosis of HLCZ01 cells in response to Hepatitis C virus infection [39].
Other host antiparasite strategies are the immune responses [40–42]. As well as apoptosis, we found that hsa-miR-3591-3p and hsa-miR-34b-5p regulate the epithelial immune responses. Several reports have shown that hsa-miR-34a regulate host immune responses in response to microbial infection. For example, hsa-miR-34a negatively regulate the innate immune response to viral infections by targeting interferon β [43]. hsa-miR-3591-3p could increases interleukin 6 mRNA levels in THP-1 cells in response to lipopolysaccharide stimulation [44]. These data suggest that hsa-miR-3591-3p, hsa-miR-34b-5p, hsa-miR-942-5p, hsa-miR-18b-3p and hsa-miR-3976 may be involved in cell apoptosis or the immune responses to C. parvum infection. Hsa-miR-34b-5p, hsa-miR-3591-3p, hsa-miR-942-5p, hsa-miR-18b-3p and hsa-miR-3976 may be involved in cell apoptosis or the immune responses to C. parvum infection.

Table 3 KEGG pathways of differentially expressed miRNAs

| Hsa-miRNA | Pathway                          | Gene                          | P value |
|----------|----------------------------------|-------------------------------|---------|
| miR-34b-5p | Ubiquitin mediated proteolysis  | CBLB, CBL, NHLRC1, BTRC, NEDD4, PIAS1 | 0.0073  |
|          | AMPK signaling pathway           | ELAVL1, RAB10, RAB14, CREB1, HNF4, APRKA2 | 0.04    |
|          | Notch signaling pathway          | APH1A, DLI, JAG1, NOTCH1, NOTCH2 | 0.017   |
|          | Hippo signaling pathway          | MOB1B, WNT2B, BTRC, BMP2R, DILG1, GSK3B | 0.013   |
| miR-942-5p | Ras signaling pathway           | RAF1, TEK, CHUK, FGF9MAPK10, MIRAS... | 0.006   |
|          | ErbB signaling pathway           | RAF1, CDKN1B, MAPK10, NRAS, PAK2, PIK3CD | 0.0082  |
|          | MAPK signaling pathway           | RAF1, TAOK1, TAB2, CACNA1E, CHUK,... | 0.014   |
|          | Wnt signaling pathway            | CREBBP, FBXW11, WNT9B, CCD1... | 0.022   |
|          | p53 signaling pathway            | CHEK1, CCD1, RARF2, SERPINE1, ZMAT3 | 0.046   |
|          | PI3K-Akt signaling pathway       | MCL1, RAF1, TEK, COL4A5, CHUK, CCND1... | 0.047   |
| miR-3591-3 | Ubiquitin mediated proteolysis  | CBLHERC3, CDC27, CUL5, MAP3K1, RFWD2... | 0.0049  |
|          | ErbB signaling pathway           | SOS2, CAMK2A, MAP2K4, NRG3, STAT5 | 0.016   |
|          | MAPK signaling pathway           | ELK4, SOS2, TAB2, DAXX, FGF14, MAP2K3... | 0.017   |
| miR-6721-5p | Cell adhesion molecules (CAMs)  | CD22, CD276, CD40LG, CD8A, CADM3, CLDN19. | 0.0018  |
|          | ErbB signaling pathway           | CDKN1A, AKT2, ELK1, SRC, CAMK2A... | 0.0037  |
|          | Ras signaling pathway            | ABL1, AKT2, BCL2L1, ELK1, GNB2, RASA4B... | 0.0087  |
|          | cGMP-PKG signaling pathway       | AKT2, ATP1A2, ATP2B2, GNA11, GNA2... | 0.018   |
| Let-7b-3p | Chemokine signaling pathway      | AKT2, CX3CR1, CRKL, CRK, GNA3, GB4... | 0.044   |
|          | actin cytoskeleton               | CDC42RKL, GNA13, ROCK1, ROCK2, SOS2... | 0.0086  |
|          | MAPK signaling pathway           | CDC42, AKT2, CRKL, CRK, MECON, RAP1B... | 0.00085 |
|          | AMPK signaling pathway           | HMGR, PFKFB3, AKT2, ELAVL1, RAB10... | 0.018   |
|          | Notch signaling pathway          | DLI1, DLI4, HES1, JAG1, JAG2, MAML2, RBPI... | 0.031   |
|          | Wnt signaling pathway            | LRP6, ROCK2, WNT5B, CTNNB1, CUL1... | 0.0085  |
|          | TGF-beta signaling pathway       | ROCK1, SP1, ACVR1, ACVR2B, BMP2R, CUL1... | 0.0085  |
|          | cGMP-PKG signaling pathway       | AKT2, ATP2B1, GNA13, GNA3, ROCK1... | 0.0036  |
| miR-454-5p | Central carbon metabolism        | AKT3, MAPK1, PDGFRA | 0.012   |
|          | Rap1 signaling pathway           | AKT3, MAPK1, PDGFRA, TLN2 | 0.016   |
| miR-1287-3p | Ras signaling pathway           | ABL2, BCL2L1, RAB5A, EFNA3, FGF12 | 0.021   |
| miR-3121-5p | Cell adhesion molecules         | CLDN2, HLA-C, NRXN1, PTPRFR, SEL, SDC1 | 0.017   |
|          | Cytokine receptor interaction    | CXCL14, KIT, RELT, TNFRSF21, ACVR1... | 0.034   |
| miR-1256 | ErbB signaling pathway           | KRAS, EGF, PIK3CG, TGFA | 0.014   |
|          | Ras signaling pathway            | KRAS, RAP1B, EGF, PIK3, CCRAC1, STK4 | 0.0093  |
|          | MAPK signaling pathway           | KRAS, MKNK2, RAP1B, TAB2, STK4... | 8.50E-5 |
|          | Rap1 signaling pathway           | KRAS, RAP1B, RAPGEF2, EGF, PIK3CG, RAC1 | 0.0068  |
| miR-4685-3p | Inflammatory mediator regulation of TRP channels | ASIC1, ADCY3, ITPR2, MAP2K6 | 0.017   |
| miR-5580-3p | ECM-receptor interaction        | COLS2A2, COL11A1, ITGA2, ITGA4, ITGA9 | 0.049   |

*P-value* refers to significance of function enrichment.
hsa-miR-942-5p, hsa-let-7b-3p, and hsa-miR-6721-5p were also identified as the key miRNAs among the miRNAs differentially expressed after *C. parvum* infection. Therefore, the functions of hsa-let-7b-3p, hsa-miR-3591-3p, hsa-miR-34b-5p, hsa-miR-942-5p, hsa-miR-6721-5p, hsa-miR-34b-3p, hsa-miR-3976, and hsa-miR-3121-5p during *C. parvum* infection require further investigation.

There were some limitations to this study that should be addressed. First, the time points in the early phase of *C. parvum* infection was relatively limited and microRNA expression profile of HCT-8 cells in the later phase of *C. parvum* infection should be further analyzed to better understand the interplay between *C. parvum* and its host at the miRNA level. Second, we only assessed microRNA regulation in a single cell line, so the results should be confirmed with primary intestinal epithelial cells or another human intestinal epithelial cells line. Last, we should decrease the dose of oocysts used to infect the monolayer, because the high dose of oocysts may potentially have triggered a stress response in the host cell, the differentially regulated miRNA could reflect such a response and be unrelated to the actual *C. parvum* infection.

Conclusions
In this study, we determined the miRNA expression profile of human intestinal epithelial cells infected by *C. parvum* for the first time, and demonstrated that the dysregulation of these miRNAs may regulate the host’s biological processes in response to *C. parvum* infection, including cell apoptosis and the immune responses. These findings extend our understanding of the miRNA regulatory network that operates during the host–*C. parvum* interaction. They also provide clues for further research into the regulatory mechanisms of miRNAs in human intestinal epithelial cells in response to *C. parvum* infection, which may identify potential targets for future *C. parvum* control strategies.

Additional file

Fig. 4 miRNA–target gene network. Network of miRNAs and their target genes at 4 h after *C. parvum* infection (a). Network of miRNAs and target genes at 12 h after *C. parvum* infection (b). Network of the key miRNAs and target genes at 12 h after *C. parvum* infection (c). In the diagram of the miRNA–gene network, the ellipses represent the miRNAs, the rectangles represent the target genes, and triangles represent the key target genes, with the interaction between them are represented by lines. The crucial miRNAs has the highest connectivity which refers to the number of edges possessed by one miRNA. The key genes are the common target genes of differentially expressed miRNAs.

Additional file 1: Figure S1. GO analysis of targets of differentially expressed miRNAs involved in the regulation of apoptotic processes and the immune response. (PDF 521 kb)

Abbreviations
DAVID: Database for annotation, visualization and integrated discovery; DMEM: Dulbecco’s modified Eagle’s medium; GO: Gene Ontology;
The authors declare that they have no competing interests.

Competing interests

This study was supported by the Key Program of the National Natural Science Foundation of China (31330079), the National Natural Science Foundation of China (31672548, 31302079), and National Key Research and Development Program of China (2017YFD0501305, 2017YFD0500405). The funding bodies had no role in the design of the study, collection, analysis, interpretation of data and in the writing of the manuscript.

Availability of data and materials

All the data supporting our findings is contained within the manuscript. Full details of the miRNA microarray analyses were deposited in the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) public database with the associated platform accession number GPL23111. The raw data are available through GEO under Accession: GSE95365. The entire miRNA data set was MIAME compliant.

Authors’ contributions

LoZ oversaw the entire project, including experimental design, reviewing the manuscript, LL and HZ edited the manuscript and performed bioinformatic analyses, RFY performed animal work and revised the manuscript, LWJ and ZC performed bioinformatic and statistical analyses and contributed intellectually and critically to the manuscript. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

This research was conducted according to the Chinese Laboratory Animal Administration Act (1988) after the review and approval of its protocol by the Research Ethics Committee of Henan Agricultural University. No specific permits were required, and no endangered or protected species were involved.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 7 February 2018 Accepted: 26 December 2018

Published online: 14 January 2019

References

1. Ryan U, Fayer R, Xiao L. Cryptosporidium species in humans and animals: current understanding and research needs. Parasitology. 2014;141(13):1667–85.
2. Hunter PR, Hadfield SJ, Wilkinson D, Lake R, Harrison FC, Chalmers RM. Subtypes of Cryptosporidium parvum in humans and disease risk. Emerg Infect Dis. 2007;13(1):82–8.
3. Ryan U, Power M. Cryptosporidium species in Australian wildlife and domestic animals. Parasitology. 2012;139(13):1673–88.
4. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchal B, Wu Y, Sow SO, Sur D, Breiman RF, et al. Burden and aetiology of diarrheal disease in infants and young children in developing countries (the global enteric multicenter study, GEMS): a prospective, case-control study. Lancet. 2013;382(9888):209–22.
5. Sniepien B. Parastic infections: time to tackle cryptosporidiosis. Nature. 2013;502(7475):189–91.
6. Bueno MJ, Pérez de Castro I, Malumbres M. Control of cell proliferation pathways by microRNAs. Cell Cycle. 2008;7(20):3143–8.
7. Kedde M, Agami R. Interplay between microRNAs and RNA-binding proteins determines developmental processes. Cell Cycle. 2008;7(7):899–903.
8. O’Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. Nat Rev Immunol. 2010;10(2):111–22.
9. Pinedo D, Azevedo-Pereira JM. The role of microRNAs in the pathogenesis of herpesvirus infection. Viruses. 2016;8(6):156–87.
10. Zhou R, Hu G, Liu J, Gong AY, Drescher KM, Chen XM. NF-kappaB p65-dependent transactivation of miRNA genes following Cryptosporidium parvum infection stimulates epithelial cell immune responses. PLoS Pathog. 2009;5(12):e1000681.
11. Staedel C, Darfeuille F. MicroRNAs and bacterial infection. Cell Microbiol. 2013;15(9):1406–507.
12. Chen XM, Splinter PL, O’Hara SP, LaRusso NF. A cellular micro-RNA, let-7i, regulates toll-like receptor 4 expression and contributes to cholangiocyte immune responses against Cryptosporidium parvum infection. J Biol Chem. 2007;282(39):28929–38.
13. Hu G, Gong AY, Roth AL, Huang BQ, Ward HD, Zhu G, Larusso NF, Hanson ND. Chen XM. Release of luminal exosomes contributes to TLR4-mediated epithelial antitumor defense. PLoS Pathog. 2013;9(6):e1003261.
14. Zhou R, Gong AY, Escheidt AN, Chen XM. miR-27b targets KRSP to coordinate TLR4-mediated epithelial defense against Cryptosporidium parvum infection. PLoS Pathog. 2012;8(5):e1002702.
15. Sato Y, Koshizuka T, Ishibashi K, Hashimoto K, Ishikawa K, Ikuta K, Yokota SI, Fuji N, Suzutani T. Involvement of herpes simplex virus type 1 UL13 protein kinase in induction of SOCS genes, the negative regulators of cytokine signaling. Microbiol Immunol. 2017;61(5):159–67.
16. Hu G, Zhou R, Liu J, Gong AY, Chen XM. MicroRNA-98 and let-7 regulate expression of suppressor of cytokine signaling 4 in bile epithelial cells in response to Cryptosporidium parvum infection. J Infect Dis. 2010;202(12):35–45.
17. Sheedy FJ, Palsson-McDermott E, Hemmessy EJ, Martin C, O’Leary JJ, Ruan Q, Johnson DS, Chen Y, O’Neill LA. Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat Immunol. 2010;11(2):141–7.
18. Verdon R, Keusch GT, Tzipori S, Grubman SA, Jefferson DM, Ward HD. An in vitro model of infection of human biliary epithelial cells by Cryptosporidium parvum. J Infect Dis. 1997;175(5):1268–72.
19. Hu M, Xia M, Chen X, Lin Z, Xu Y, Ma Y, Su L. MicroRNA-141 regulates Smad signaling of epithelial cell immune responses. PLoS Pathog. 2012;8(5):e1002702.
20. Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, Jones PA. Specific activation of microRNA-427 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell. 2006;9(6):43–53.
21. Li R, Qian N, Tao K, You N, Wang X, Dou K. MicroRNAs involved in neoplastic transformation of liver cancer stem cells. J Exp Clin Cancer Res. 2010;29:169.
22. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2–Delta Delta C(T) method. Methods. 2001;25(4):402–8.
23. Agarwal V, Bell GW, Nam J, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. elife. 2015;4:e05005.
24. Weng N, Wang X, miRDB: an online resource for microRNA target prediction and functional annotations. Nucleic Acids Res. 2015;43(D1):4–50.
25. Huang d W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools:kingdom of genes and genomes; SOCS: Suppressor of cytokine signaling.

KEGG: Kyoto encyclopedia of genes and genomes; SOCS: Suppressor of cytokine signaling.
28. Mokutani Y, Uemura M, Munakata K, Okuzaki D, Haraguchi N, Takahashi H, Nishimura J, Hata T, Murata K, Takemasa I, et al. Down-regulation of microRNA-132 is associated with poor prognosis of colorectal Cancer. Ann Surg Oncol. 2016;23(5):599–608.

29. Ji J, Shi J, Budhu A, Yu Z, Forgues M, Roessler S, Amb S, Chen Y, Meltzer PS, Croce CM, et al. MicroRNA expression, survival, and response to interferon in liver cancer. N Engl J Med. 2009;361(15):1437–47.

30. Li J, Tan S, Kooger R, Zhang C, Zhang Y. MicroRNAs as novel biological targets for detection and regulation. Chem Soc Rev. 2014;43(2):506–17.

31. Chen XM, Levine SA, Tietz P, Kruiger E, McNiven MA, Jefferson DM, Mahle M, LaRusso NF. Cryptosporidium parvum is cytopathic for cultured human biliary epithelia via an apoptotic mechanism. Hepatol. 1998;28(4):906–13.

32. McCole DF, Eckmann L, Laurent F, Kagnoff M. Intestinal epithelial cell apoptosis following Cryptosporidium parvum infection. Infect Immun. 2000;68(3):1710–3.

33. Chen XM, Gores GJ, Paja CV, LaRusso NF. Cryptosporidium parvum induces apoptosis in biliary epithelia by a Fas/Fas ligand-dependent mechanism. Ann J Phys. 1999;277(1):599–608.

34. Widmer G, Corey EA, Stein B, Griffiths JK, Tzipori S. Host cell apoptosis impairs Cryptosporidium parvum development in vitro. J Parasitol. 2003;89(5):922–8.

35. Liu J, Deng M, Lancto CA, Abrahamson MS, Rutherford MS, Enomoto S. Biphasic modulation of apoptotic pathways in Cryptosporidium parvum infected human intestinal epithelial cells. Infect Immun. 2009;77(2):837–49.

36. Cai Y, Chen H, Jin L, You Y, Shen J. Toxoplasma gondii inhibits apoptosis via a novel STAT3-miR-17-92-Bim pathway in macrophages. Cell Signal. 2014;26(6):1204–12.

37. Cai Y, Chen H, Jin L, You Y, Shen J. STAT3-dependent transactivation of miRNA genes following Toxoplasma gondii infection in macrophage. Parasite Vectors. 2013;6:356.

38. Fan N, Wang J. MicroRNA 34a contributes to virus-mediated apoptosis through binding to its target gene Bax in influenza virus infection. Biomed Pharmacother. 2016;81:1464–70.

39. Yang D, Meng X, Xue B, Liu N, Wang X, Zhu H. miR-942 mediates hepatitis C virus-induced apoptosis via regulation of IGF12a. PLoS One. 2014;9(4):e94501.

40. McDonald V, Korbel DS, Barakat FM, Choudhry N, Petry F. Innate immune responses against Cryptosporidium parvum infection. Parasite Immunol. 2013;35(2):55–64.

41. Pantenburg B, Dann SM, Wang HC, Robinson P, Castellanos-Gonzalez A, Lewis DE, White AC Jr. Intestinal immune response to human Cryptosporidium sp. infection. Infect Immun. 2008;76(1):23–9.

42. Petry F, Jakobi V, Tessema TS. Host immune response to Cryptosporidium parvum infection. Exp Parasitol. 2010;126(3):304–9.

43. Witwer KW, Sisk JM, Gama L, Clements JE. MicroRNA regulation of IFN-beta protein expression: rapid and sensitive modulation of the innate immune response. J Immunol. 2010;184(5):2369–76.

44. Woo MY, Yun SJ, Cho O, Kim K, Lee ES, Park S. MicroRNAs differentially expressed in Behçet disease are involved in interleukin-6 production. J Inflamm. 2016;13:22.