IncRNA028466 Regulates Th1/Th2 Cytokines Expression and Associated with *Echinococcus Granulosus* Antigen P29 Immunity

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Abstract

Background: *Cystic echinococcosis* (CE) is a parasite disease that was caused by *Echinococcus granulosus* (Eg). The recombinant *Echinococcus granulosus* antigen P29 (rEg.P29) has been shown to conferred effective immunity to sheep and mice during *Echinococcus granulosus* secondary infection in our previous study. In our study, we strove to research the ability of Long noncoding RNA 028466 (lncRNA028466) as a regulator for the protective immunity mediated by rEg.P29 vaccination and studied the effects of lncRNA028466 on CD4⁺T cells differentiation in mice spleen.

Methods: Female BALB/c mice were divided into two groups and were vaccinated subcutaneously with rEg.P29 antigen and PBS as a control (12 mice each group). Following prime-boost vaccination, CD4⁺T, CD8⁺T and B cells from the spleen were isolated by flow cytometry. Quantitative real-time PCR (qRT-PCR) was performed to measure the expression of lncRNA028466 in these three kinds of cells. Then, lncRNA028466 was over-expressed and knock-downed. Furthermore, were d by qRT-PCR and ELISA were performed to evaluate the production of IFN- γ , IL-2, IL-4, and IL-10, as well as was flow cytometry was performed to detect the differentiation of Th1 and Th2 subgroups.

Results: lncRNA028466 was significantly decreased after the second-week of immunization with rEg.P29 antigen. The proportion of CD4⁺T cells was increased after rEg.P29 immunization. Subsequent overexpression and knockdown of lncRNA028466 in naive CD4⁺T cells were performed to detect changes in Th1 and Th2 cytokines expression. Overexpression of lncRNA028466 facilitated the production of IL-4, IL-10 and suppressed the production of IFN- γ , IL-2. Furthermore, after transfection with siRNA028466, IL-2 production was facilitated and IL-10 production was suppressed in naive CD4⁺T cells.

Conclusions: Immunization with rEg.P29 down-regulated the expression of lncRNA028466, which was related to a higher Th1 immune response, a higher Th2 immune response with lncRNA028466 overexpression, a higher IL-2 mRNA expression, and a lower IL-10 mRNA expression with lncRNA028466 knockdown. Our results suggest that lncRNA028466 may be involved in rEg.P29-mediated immune response by regulating cytokines expression of Th1 and Th2.

Background

CE, also named hydatid disease, is a worldwide chronic parasitic disease, threatening the health of livestock and humans[1]. Human beings are exposed to the eggs of *E. granulosus* after close contact with infected livestock or contaminated food[2, 3]. At present, diagnosis of human echinococcosis mainly dependent on imaging techniques. The pathogen in the human body is relatively long, ranging from 1 to 30 years and the symptoms of the initial infection is not obvious. So, it is hard to diagnose, treat, and control[4-6]. Due to the limitations of current diagnostic and therapeutic measures, a mass of research has been done in the field of immunology associated with *E. granulosus* infection in the hope of developing future vaccines[7, 8]. Vaccination would be one of the effective preventive healthcare against

echinococcosis[1, 9]. Due to the complicated host-parasite relationship[10], there is still no commercialized vaccine.

By far, there have been some effective antigens as vaccine candidates for prevention CE. Vaccination of intermediate hosts of *E. granulosus* with the Eg95 antigen has induced markable protective efficacy[4, 11]. Another study[12]documented the P29 protein as a 29-kDa antigen, which has been characterized by *E. granulosus* protoscolex-derived soluble somatic antigen[13, 14]. Our previous studies confirmed that vaccination of rEgP29 in sheep and mice carrying infection of *E. granulosus* eggs had resulted in remarkable protective efficacy and induced Th1 cell to produce IFN- γ against *E. granulosus* infection[14, 15]. However, the mechanism of rEgP29 antigen influenced the host immune response and regulated the differentiation of CD4⁺ T cells is still unclear.

Understanding the immune response between host and parasite is necessary[16]. The role of CD4⁺T cells in bacterial, viral, and parasitic infections and the adaptive immune system is important [17-19]. The distinguishing feature of the immune response caused by *E. granulosus* is that cellular (Th1) and humoral (Th2) immune response coexist[20]. It is commonly accepted that in most parasitic diseases Th1- or Th2-type response can be able to control pathogens, early Th1 cells activation confers host protective immunity, while Th2 cell activation is related to the progression of the chronic stage when infected with *E. granulosus*[21, 22]. Changes in the immune response from Th2 to Th1 seem to be required for a protective phenotype[23]. A polarized cytokine response plays an important role in some parasitic diseases where Th1- or Th2-type reactions are associated with susceptibility or resistance[24, 25]. Thus, understanding what impels cytokines expression toward different patterns contributes to the immune therapy and vaccination scheme[26].

lncRNAs act as pivotal regulators of gene expression in both innate and adaptive immune responses and play a crucial role in important cellular processes, like differentiation and activation of lymphocytes[27, 28]. Distinct lncRNA expressions were identified from early T cells to terminal T helper (Th) cells suggesting that lncRNA could modulate the development and differentiation of T cells[29]. Consistent with this finding, lncRNA TMEVPG1 was specifically expressed in Th1 cells and lncR-Ccr2-50AS was proved to regulate Th2 cell differentiation[30, 31]. The ability of lncRNA to regulate CD4⁺T cell differentiation could have crucial implications for CE where a Th2 predominant immune response generates IL-4 and IL-10 cytokines[32, 33]. Although lncRNAs have a variety of biological functions, the knowledge of the potential biological functions of lncRNAs in parasitic infection is still vast and largely unexplored[34, 35]. A recent study found that microRNA (miRNA), lncRNA, and circular RNA (circRNA) had been identified in protoscoleces exosome-like vesicles and hydatid fluid, and was predicted to participate in the parasite-host interactions[36]. Therefore, identifying how lncRNA regulates CD4⁺T cell differentiation could provide new mechanistic insights and therapeutic targets for CE.

We used microarray analysis to investigate the lncRNA expression profiles in splenic lymphocytes from rEg.P29 immunization of mice with secondary infection of *E. granulosus*, bio-information analysis showed a significant decrease in lncRNA028466 [37]. Furthermore, in vitro, we demonstrated that

lncRNA028466 was markedly downregulated in CD4⁺T of spleens from mice vaccinated with r*Eg*.P29 antigen. The purpose of our study was to research the influence of lncRNA028466 on the expression of cytokines-producing Th1 and Th2 cells. It is hoped that our datasets provide an important resource for future studies of the vaccine of r*Eg*.P29 and the function of lncRNA in host-parasite interaction.

Methods

Mice

We obtained six to eight weeks female BALB/c mice from the Animal Laboratory Center of Ningxia Medical University (SCXK2018-0004). The mice were raised in pathogen-free conditions, with eight groups of mice in each cage and fed clean food and water. All mouse experiments were given permission by the Ningxia Medical University Institutional Review Committee and carried out in strict accordance with national and institutional guidelines.

r*Eg*.P29 antigen preparation and vaccination protocols

The recombinant plasmid is preserved in our laboratory. Then we converted recombinant plasmid P29/pET28a into *E.coli* BL21 pLysS and mixed them with LB liquid medium for incubation, which induced expression at 37 °C 200 r/min for 5 h, under the condition of 1 mol/mL isopropyl-β-d-sulfur semi-lactose glycoside (IPTG, Invitrogen). Subsequently, his6-tagged r*Eg*P29 was purified by nickel chelation affinity (Novagen) following the manufacturer's protocol. Western blot was performed to detect the r*Eg*.P29 purity. Next, we randomly classified twenty-four mice into two groups of twelve mice as immune and control groups. The immune group was injected subcutaneously with 10 μg purified r*Eg*.P29 protein at three points in the abdomen. The r*Eg*.P29 protein is emulsified with phosphate buffer saltwater (PBS) and Freund's adjuvant in a volume of 100 μL for two times. Primary immunity used Freund's complete adjuvant, and 2 weeks after the initial immunity, Freund's incomplete adjuvant was used to strengthen immunity. The control group was injected with an equal volume of PBS. 2 weeks after the booster immunization, mice were used to do later experiments.

Western blot analysis

The BCA Protein Assay Kit (Beyotime Biotechnology) was used to measure the concentration of r*Eg*.P29. Then, the r*Eg*.P29 protein was boiled at 100 °C, 10 min, and 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to isolate the protein. PVDF membrane (Solarbio Science Technology, Beijing, CN) was used to transfer the r*Eg*.P29 protein. 5% skim milk was used to enclose the PVDF membrane for 2 h under room temperature, immune mice and control mice serum (1:500 dilution) were used to incubate the membrane overnight at 4 °C. Next, the second antibody Goat Anti-Mouse IgG/HRP (Solarbio) was used to incubate the membrane for 1 h under room temperature. At last, 3,3'-Diaminobenzidine Tetrahydrochloride Horseradish Peroxidase Color Development Kit (Solarbio) was used to detect the r*Eg*.P29 protein.

CD4⁺T, CD8⁺T, and B cells sorting

Following booster immunization, mice were treated by cervical dislocation. All spleen and lymphocyte operations were carried out under sterile conditions. Spleens were removed from the two groups respectively. Subsequently, the single-cell suspension was prepared and then spleen tissue lymphocyte isolation medium kit (Tianjin Hao Yang Biological Manufacture, Tianjin, China) was used to isolate spleen lymphocyte cell following the manufacturer's protocol. Finally, we diluted the cell concentration to 1×10^7 / mL using a 10% FBS (Gibco, Grand Island, USA) PBS solution and divided into three groups, 300 μ L / tube. Furthermore, stained for cell-surface markers, including APC-labeled Anti-CD4 (552051), Percp-cy5.5-labeled Anti- CD3e(562286), PE-labeled Anti-CD8 (558106) FITC-labeled Anti-CD19 (1575-02S) (BD Biosciences, San Jose, CA, USA), placed at 4 °C in the dark for 30 min. After washing twice, cells were suspended using 2 mL PBS with 10% FBS and sorted by flow cytometry. Finally, collecting CD4⁺T, CD8⁺T, and B cells used to do later experiment.

lncRNA028466 overexpression lentivirus construction and siRNA synthesis

The endotoxin-free plasmid of lncRNA028466 was extracted using an endotoxin-free plasmid extract kit (Beijing Tiangen Biochemical Technology company, Beijing, China) according to the manufacture's instruction. 293T packing cells (Thermo Fisher Scientific, CN.) were cultured with 8 mL DMEM medium (Invitrogen) with 10 % FBS (Gibco), 1% 100 x penicillin-streptomycin solution (Invitrogen) in the condition of 37 °C and 5% CO₂ before transfection. When the cell density reached 80 % - 90 %, 293T cells were transfected with 9 μ g pCDH-028466, 9 μ g pLP1, 9 μ g pLP2 and 9 μ g pLP VSV-G (Invitrogen) in the presence of Polyjet (Signa Gen, USA) to package the recombinant lentivirus. After 48 h, the supernatant that contained virus was collected under 50,000 g 4 °C and virion was collected after 2 h.

Three small interfering RNAs (siRNAs)–targeted separate sequences of lncRNA028466 were synthesized by Tianjin Sheweisi Biotechnology Company, including siRNA1, siRNA2 and siRNA3.

siRNA-1:

GGUUGAGAUUGGACGUUUCAUDTDT(sense) AUGAAACGUCCAAUCUCAACCDTDT(antisense)

siRNA-2:

GCCUAACCUUGUACCCUCUUUDTDT(sense) AAAGAGGGUACAAGGUUAGGCDTDT(antisense)

siRNA-3:

GGCCAGAUAAAGCUGCAADTDT (sense)

UUGCAGCUCUUAUCUGGCCDTDG(antisense)

Naive CD4⁺T cells sorting, transfection and differentiation

Healthy female BALB/c mouse spleen was placed on a 70 µm nylon cell filter to prepare single-cell suspension. Briefly, an magnetic beads isolation kit (Miltenyi Biotech, Bergisch Gladbach, Germany) was used for negatively selecting naive CD4⁺T cells following the manufacturer’s instruction. The purification of naive CD4⁺T cells population (CD4⁺CD25⁻CD62L^{hi}CD44^{low}) was 90% . Subsequently, RPMI 1640 (Gibco, Grand Island, USA) with 2 % FBS was used for resuspending naive CD4⁺T cells, and then the cells was cultivated at 37 °C, 5% CO₂ in 1´10⁶ cells / well of 48-well plate, then 100 uL pCDH-CMV and pCDH-028466 lentivirus or 20 nM siRNA and negative control siRNA were added in the presence of Lipofectamine 3000 (Thermo Fisher Scientific, CN.), total volume was 250µL / well. After 6 h, the transfected cells were removed into a 24-well plate. For Th1 and Th2 cells activation, as previous reported[38]. The cells were used to do later experiment after transfection 96 h.

Quantitative real-time PCR

Cultures were harvested after 96 h. Trizol reagent (Invitrogen) was used for isolating the total RNA. Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, CN.) was used to synthesize cDNA according to the manufacturer’s instruction. SYBR Green probes (DBI Bioscience) and Step One Plus TM Real-Time PCR system were used to analyze Th1 cytokines IFN-γ, IL-2, and Th2 cytokines IL-4, IL-10 expression. The gene relative exprerssion were calculated according to the 2^{-ΔΔCt} method. Specific primer sequences were listed in Table 1.

Table 1. Sequences of primers used for qRT-PCR

Gene	primer5'→3'
lncRNA 028466	FAGGCCCAAGTCTCTCTTGTGA RGGGTCTGATGGGTCTCAT
IL-2	FTGAGCAGGATGGAGAATTACAGG RGTCCAAGTTCATCTTCTAGGCAC
IFN-γ	FCTCCCGTGGCTTCTAGTGC RGCCTTAGTTTGGACAGGATCTG
IL-4	FATCATCGGCATTTTGAACGAGG RTGCAGCTCCATGAGAACACTA
IL-10	FGCCACGGCACAGTCATTGA RTGCTGATGGCCTGATTGTCTT
GAPDH	FAGGTTCGGTGTGAACGGATTTG RGGGGTCGTTGATGGCAACA

Flow cytometry analysis

We cultured the transfected cells under Th1 and Th2 polarizing conditions. Cell Stimulation Cocktail and Protein Transport Inhibitor (Invitrogen) were added into transfected cells in the last 6 h incubation. For cell surface staining, we first used 0.1 % BSA, 0.05 % sodium azide PBS buffer washed the collected cells. Then, the surface antibody APC-labeled anti-CD4 and PerCP-cy-5.5-labeled anti-CD3 (BD Biosciences) were used to stain the cells under the dark for 30 min at 4 °C. Subsequently, 4 % paraformaldehyde was used to fix the cells under the room temperature for 8 min in dark, and 0.1 % BSA, 0.05 % sodium azide, and 0.1 % saponin (Sigma) PBS buffer was used to permeabilize the cells under the room temperature for 2 h in dark. After washing, PE-labeled anti-IFN- γ , PE-labeled anti-IL-2, PE-labeled anti-IL-4, PE-labeled anti-IL-10 (BD Biosciences) were respectively used to stained the cells under the dark for 30 min at 4 °C. Stained cells were assayed with flow cytometry (BD Biosciences) and the program FlowJo version 10.0 (Tree Star Inc. USA) was used to analyze the data.

ELISA

Transfected cells were stimulated with Cell Stimulation Cocktail in the last 6 h incubation. Supernatants were collected after 96 h and specific ELISA kit (BD Biosciences, San Jose, CA, USA) was used to detect the cytokines levels following the manufacturer's protocol.

Statistical analysis

Using SPSS 17.0 statistical software and GraphPad Prism6.0 software to analyze the experimental results, the measurement data represented as mean $\pm$ standard error, according to comparison between the two groups using two-tailed t test. The test level was $\alpha = 0.05$.

Results

Microarray analysis reveals significant down-regulation of lncRNA028466 transcripts in spleen lymphocytes of mice immunized with rEg.P29 antigen

First, we detected a high purity expression band of rEg.P29 antigen at 31000 Mr through Western blot (Fig.1a). Microarray analysis revealed that lncRNA028466 was down-regulated 2.31-fold in spleen lymphocytes of mice immunized with rEg.P29 antigen. To verify the expression of lncRNA028466 in spleen lymphocytes following immunization, qRT-PCR was used to determine the down-regulation of lncRNA028466. lncRNA028466 exhibited a 1.87-fold-lower expression ($t = 7.906$, $P = 0.0002$) compared with that in the control group (Fig.1b).

The expression of lncRNA028466 is decreased in CD4⁺T of spleens from mice immunized with rEg.P29 antigen

Because lncRNA028466 expression decreased in spleen lymphocytes of mice immunized with rEg.P29 antigen, we aim to understand the role that lncRNA028466 plays in rEg.P29-mediated immune response.

We then respectively isolated splenic CD4⁺T, CD8⁺T and B cells from the control and immune group using flow cytometry. The percentage of CD4⁺T, CD8⁺T, and B cells were respectively 21.6%, 7.1%, 56.7% in the control group (Fig.2a) and 26%, 8.0%, 56.0% in the immune group (Fig.2b). The data indicated that CD4⁺T cells were increased following immunization with rEg.P29. Furthermore, qRT-PCR was performed to measure the expression of lncRNA028466 in CD4⁺T, CD8⁺T, and B cells. The expression of lncRNA028466 in CD4⁺T cells from immune group was 1.68 times lower ($t = 9.651$, $P = 0.001$) than the control group (Fig.2c), however, there were no prominent changes in CD8⁺T and B cells (Fig.2d, e). All in all, these data suggest that lncRNA028466 could have an effect on cellular immune responses associated with rEg.P29 antigen immunity.

Overexpression of lncRNA028466 biases naive CD4⁺T cell differentiation toward the Th2 phenotype

To understand the role that lncRNA028466 play in CD4⁺T cell differentiation, we isolated splenic naive CD4⁺T lymphocytes. In addition, we used 293T cells packing lncRNA028466 overexpression lentivirus (pCDH-028466, GFP) and empty vector (pCDH-CMV, GFP). The transfection efficiency of transfection naive CD4⁺T cells with pCDH-028466 and pCDH-CMV was detected by qRT-PCR analysis, lncRNA028466 overexpression lentivirus exhibited a 28.56-fold-higher expression ($t = 7.924$, $P = 0.0005$) compared with empty vector (Fig.3a). The result suggested that lncRNA028466 overexpression lentiviral vector was successfully transfected into the cells. Subsequently, naive CD4⁺T cells were infected with pCDH-CMV and pCDH-028466. Then, the expression of Th1-related cytokines IL-2, IFN- γ , and Th2-related cytokines IL-10, IL-4 were detected respectively from mRNA and extracellular levels by qRT-PCR and ELISA. As a result, according to qRT-PCR analysis, overexpression of lncRNA028466 inhibited the expression of IFN- γ ($t = 7.564$, $P = 0.0003$) and IL-2 ($t = 13.28$, $P = 0.001$) (Fig.4b,c) and increased the expression of IL-4 ($t = 4.157$, $P = 0.006$) and IL-10 ($t = 7.367$, $P = 0.0003$) (Fig.4d,e). The result was confirmed by ELISA analysis (Fig.4f-i).

Considering the result that overexpression of lncRNA028466 upregulated Th2-related cytokines IL-4 and IL-10 expression, and downregulated Th1-related cytokines IFN- γ and IL-2 expression, we studied the role of lncRNA028466 in CD4⁺T cell differentiation. pCDH-CMV and pCDH-028466 were transfected into naive CD4⁺T cells, which cultured in the Th1 and Th2 cell-polarizing conditions. The FACS analysis showed that lncRNA028466 overexpression lentivirus transfected cells increased differentiation towards IL-4- (Fig.4c) and IL-10- (Fig.4d) producing Th2 subtype, compared with empty vector group. However, there was a remarkable reduction in IFN- γ - (Fig.4a) and IL-2-producing (Fig.4b) Th1 cells. All in all, these results may indicate that overexpression of lncRNA028466 biases naive CD4⁺T cell differentiation toward the Th2 phenotype.

Knockdown of lncRNA028466 promotes naive CD4⁺T cell differentiation toward the Th1 subgroup

To confirm if lncRNA028466 contributes to CD4⁺T cell differentiation, we designed siRNA to knock down its expression in naive CD4⁺T cells. For siRNA interference efficiency, siRNA1, siRNA2, and siRNA3 were

respectively transfected into naive CD4⁺T cells, and qRT-PCR was performed. According to the result (Fig.5a), siRNA1 efficiently knocked down lncRNA028466 by 5.88-fold ($t = 17.98$, $P \leq 0.001$) compared with negative siRNA and was used to later experiment. Subsequently, siRNA1 and negative siRNA were transfected into naive CD4⁺T cells, qRT-PCR and ELISA were performed to measure Th1-related cytokines IL-2, IFN- γ , and Th2-related cytokines IL-10, IL-4 expression. We found that knockdown of lncRNA028466 upregulated IL-2 ($t = 14.10$, $P \leq 0.0001$) production (Fig.5c, g) and downregulated IL-10 ($t = 6.822$, $P = 0.0005$) production (Fig.5e, i). There was a mild increase of IFN- γ (Fig.5b, f), and a mild decrease of IL-4 (Fig.5d, h), however, the levels of IFN- γ and IL-4 did not reach statistical significance. Maybe, the low levels of mRNA and extracellular expression led to undetected.

Similarly, siRNA transfected naive CD4⁺T cells were placed in the Th1 and Th2 cell-polarizing conditions. The FACS analysis showed siRNA transfected cells differentiated towards IFN- γ - (Fig.6a) and IL-2-producing (Fig.6b) Th1 subtype, compared with negative control group. However, there was a remarkable reduction in the Th2-related cytokines IL-4 (Fig.6c) and IL-10 (Fig.6d). Taken together, the results indicate that knockdown of lncRNA028466 promotes naive CD4⁺T cells differentiation toward the Th1 phenotype.

Discussion

The purpose of our study was to research the contribution of lncRNA028466 to the differentiation of splenic CD4⁺T cells. CD4⁺T cells are major participators in CE, and the bias of CD4⁺T cells towards Th2 subgroup causes an unbalanced secretion of cytokines producing Th1 and Th2 subgroups, which resulted in the chronic progression of CE[39, 40]. Consistent with this theory, in our study, the proportion of CD4⁺T cells was augmented following immunization with rEg.P29. This means that CD4⁺T cells participate in the immunoreaction associated with rEg.P29.

The expression of lncRNA was characterized by lineage specific, and played crucial roles during CD4⁺T cell differentiation towards Th1 and Th2 cells[29, 41, 42]. At present, the relevance of lncRNA to the pathogenesis, host resistance and parasite evasion during parasitic infection is emphasized[35]. There is increasing attention to the contribution of lncRNA to *E. granulosus* infection[36]. This kind of research will contribute to the reasonable design of immune therapy and vaccination scheme. In the present study, we discovered that lncRNA028466 was significantly decreased in CD4⁺T cells following rEg.P29 immunization. On the one hand, overexpression of lncRNA028466 not only enhanced differentiation of Th2 cells but also promoted the secretion of Th2-related cytokines IL-10 and IL-4, meanwhile, suppressed the expression of Th1-related cytokines IFN- γ and IL-2. On the other side, knockdown of lncRNA028466 inhibited differentiation of Th2 cells and heightened differentiation of Th1 cells. Based on this finding, we propose that lncRNA028466 may participate in the protective rEg.P29-mediated immunity through modulating Th1 and Th2 cytokines expression.

The polarization of Th1 and Th2 subgroups is a crucial determinant of whether the response to the pathogen will confer protection to the host or exacerbate the disease[25]. IFN- γ is demonstrated to inhibit *Echinococcus* activity and contributes to the formation of the protective immunity mediated by Th1 cells

during *E. granulosus* infection[26, 40]. Mouse experimental studies supporting IL-4- and IL-10-mediated immunosuppression may be key to parasite evasion and survival[20, 43]. The study demonstrated that the increased Th1 immune response was related to lower IL-4 and IL-10 expression, as well as lncRNA028466 overexpression. The immunoprotection of rEg.P29 may be realized by lncRNA028466 regulating the expression of cytokines associated with Th1 and Th2 cells. However, the mechanism by which lncRNA028466 regulates cell differentiation in Th1 and Th2 needs further study.

Conclusions

In conclusion, lncRNA028466 may be involved in rEg.P29 vaccination-mediated Th1 protective immunity. The study of the function of lncRNA in T helper responses is an area that needs to be explored, which will help deepen our learning about the complicated immune response. We expect that further characterization of the identified lncRNA028466 will reveal important role of lncRNA in host-parasite interaction and CD4⁺T cell differentiation.

Abbreviations

Eg: *Echinococcus granulosus*; lncRNA: long non-coding RNA; rEg.P29: recombinant *Echinococcus granulosus* antigen P29; PBS: Phosphate buffered solution; IPTG: Isopropyl β-D-Thiogalactoside; FBS: Fetal bovine serum; DMEM: Dulbecco Modified Essential Medium; RPMI1640: Roswell Park Memorial Institute; qRT-PCR: Quantitative real time polymerase chain reaction; Treg: Natural regulatory cell; BSA: Bovine serum albumin.

Declarations

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Availability of data and materials

This study-related data were obtained from the corresponding author.

Authors' contributions

WZ was in charge of the project design; CW and SY were responsible for conducting the experiments, the data analysis, and paper writing; XD and JT was responsible for designing and synthesizing relevant primers in the experiment; JY was responsible for flow cytometry sorting of lymphocyte subsets; YW was

in charge of the revision; MZ and NN were responsible for the experimental guidance; All authors read and approved the final manuscript.

Ethics approval and consent to participate

All mouse experiments were given permission by the Ningxia Medical University Institutional Review Committee (Permit number: SCXK2018-0004) and carried out in strict accordance with national and institutional guidelines. The data obtained met the

appropriate ethical requirements.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

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References

1. Anvari D, Rezaei F, Ashouri A, *et al*. Current situation and future prospects of Echinococcus granulosus vaccine candidates: A systematic review. *Transbound Emerg Dis* 2020; **6**.
2. Li Z, Zhang C, Li L, *et al*. The local immune response during Echinococcus granulosus growth in a quantitative hepatic experimental model. *SCI REP-UK* 2019; **9**: 19612.
3. Moro P, Schantz PM. Echinococcosis: a review. *INT J INFECT DIS* 2009; **13**: 125-133.
4. Wen H, Vuitton L, Tuxun T, *et al*. Echinococcosis: Advances in the 21st Century. *CLIN MICROBIOL REV* 2019; **32**: e18-e75.

5. Li Z, Zhang C, Li L, *et al.* The local immune response during *Echinococcus granulosus* growth in a quantitative hepatic experimental model. *SCI REP-UK* 2019; **9**: 19612.
6. Khanna A, Kumar J, Vargas MA, *et al.* A genome-wide screen of bacterial mutants that enhance dauer formation in *C. elegans*. *SCI REP-UK* 2016; **6**: 38764.
7. Wang L, Gao J, Lan X, *et al.* Identification of combined T-cell and B-cell reactive *Echinococcus granulosus* 95 antigens for the potential development of a multi-epitope vaccine. *Annals of Translational Medicine* 2019; **7**: 652.
8. Larrieu E, Gavidia CM, Lightowlers MW. Control of cystic echinococcosis: Background and prospects. *ZOO NOSES PUBLIC HLTH* 2019; **66**: 889-899.
9. Heath DD, Holcman B. Vaccination against echinococcus in perspective. *ACTA TROP* 1997; **67**: 37-41.
10. Zhang Q, Ye J, Zheng H. Dexamethasone attenuates echinococcosis-induced allergic reactions via regulatory T cells in mice. *BMC IMMUNOL* 2016; **17**: 4.
11. Larrieu E, Herrero E, Mujica G, *et al.* Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: Early impact and preliminary data. *ACTA TROP* 2013; **127**: 143-151.
12. González G, Spinelli P, Lorenzo C, *et al.* Molecular characterization of P-29, a metacestode-specific component of *Echinococcus granulosus* which is immunologically related to, but distinct from, antigen 5. *MOL BIOCHEM PARASIT* 2000; **105**: 177-185.
13. Boubaker G, Gottstein B, Hemphill A, Babba H, Spiliotis M. *Echinococcus* P29 antigen: molecular characterization and implication on post-surgery follow-up of CE patients infected with different species of the *Echinococcus granulosus* complex. *PLOS ONE* 2014; **9**: e98357.
14. Shi Z, Wang Y, Li Z, *et al.* Cloning, expression, and protective immunity in mice of a gene encoding the diagnostic antigen P-29 of *Echinococcus granulosus*. *ACTA BIOCH BIOPH SIN* 2009; **41**: 79-85.
15. Wang H, Li Z, Gao F, *et al.* Immunoprotection of recombinant Eg.P29 against *Echinococcus granulosus* in sheep. *VET RES COMMUN* 2016; **40**: 73-79.
16. Siracusano A, Delunardo F, Teggi A, Ortona E. Cystic echinococcosis: aspects of immune response, immunopathogenesis and immune evasion from the human host. *Endocr Metab Immune Disord Drug Targets* 2012; **12**: 16-23.
17. Tubo NJ, Jenkins MK. CD4⁺ T Cells: guardians of the phagosome. *CLIN MICROBIOL REV* 2014; **27**: 200-213.
18. Rogan MT. T-cell activity associated with secondary infections and implanted cysts of *Echinococcus granulosus* in BALB/c mice. *PARASITE IMMUNOL* 1998; **20**: 527-533.
19. Pang N, Zhang F, Li S, *et al.* TGF- β /Smad signaling pathway positively up-regulates the differentiation of Interleukin-9-producing CD4⁺ T cells in human *Echinococcus granulosus* infection. *J INFECTION* 2018; **76**: 406-416.
20. Amri M, Mezioug D, Touil-Boukoffa C. Involvement of IL-10 and IL-4 in evasion strategies of *Echinococcus granulosus* to host immune response. *EUR CYTOKINE NETW* 2009; **20**: 63.

21. Dorosti Z, Tolouei S, Khanahmad H, *et al.* IL-4 gene expression in adventitial layer (fibrous layer) of hepatic ovine and bovine hydatid cysts. *Journal of Parasitic Diseases* 2016; **40**: 855-859.
22. Siracusano A, Delunardo F, Teggi A, Ortona E. Host-parasite relationship in cystic echinococcosis: an evolving story. *Clin Dev Immunol* 2012; **2012**: 639362.
23. Gabaglia CR, Valle MT, Fenoglio D, Barcinski MA, Manca F. Cd4(+) T cell response to *Leishmania* spp. in non-infected individuals. *HUM IMMUNOL* 2000; **61**: 531-537.
24. Maizels RM, Bundy DA, Selkirk ME, Smith DF, Anderson RM. Immunological modulation and evasion by helminth parasites in human populations. *NATURE* 1993; **365**: 797-805.
25. Jankovic D, Liu Z, Gause WC. Th1- and Th2-cell commitment during infectious disease: asymmetry in divergent pathways. *TRENDS IMMUNOL* 2001; **22**: 450-457.
26. Corrêa F, Hidalgo C, Stoore C, Jiménez M, Hernández M, Paredes R. Cattle co-infection of *Echinococcus granulosus* and *Fasciola hepatica* results in a different systemic cytokine profile than single parasite infection. *PLOS ONE* 2020; **15**: e238909.
27. Chen YG, Satpathy AT, Chang HY. Gene regulation in the immune system by long noncoding RNAs. *NAT IMMUNOL* 2017; **18**: 962-972.
28. Pagani M, Rossetti G, Panzeri I, *et al.* Role of microRNAs and long-non-coding RNAs in CD4(+) T-cell differentiation. *IMMUNOL REV* 2013; **253**: 82-96.
29. Hu G, Tang Q, Sharma S, *et al.* Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation. *NAT IMMUNOL* 2013; **14**: 1190-1198.
30. Vigneau S, Rohrlisch PS, Brahic M, Bureau JF. Tmevpg1, a candidate gene for the control of Theiler's virus persistence, could be implicated in the regulation of gamma interferon. *J VIROL* 2003; **77**: 5632-5638.
31. Heward JA, Lindsay MA. Long non-coding RNAs in the regulation of the immune response. *TRENDS IMMUNOL* 2014; **35**: 408-419.
32. Rostami-Rad S, Jafari R, Yousofi Darani H. Th1/Th2-type cytokine profile in C57 black mice inoculated with live *Echinococcus granulosus* protoscolices. *J INFECT PUBLIC HEAL* 2018; **11**: 834-839.
33. Riganò, Profumo, Buttari, Teggi, Siracusano. Cytokine gene expression in peripheral blood mononuclear cells (PBMC) from patients with pharmacologically treated cystic echinococcosis. *CLIN EXP IMMUNOL* 1999; **118**: 95-101.
34. Yu A, Wang Y, Yin J, *et al.* Microarray analysis of long non-coding RNA expression profiles in monocytic myeloid-derived suppressor cells in *Echinococcus granulosus*-infected mice. *PARASITE VECTOR* 2018; **11**: 327.
35. Menard KL, Haskins BE, Denkers EY. Impact of *Toxoplasma gondii* Infection on Host Non-coding RNA Responses. *FRONT CELL INFECT MI* 2019; **9**: 132.
36. Zhang X, Gong W, Cao S, *et al.* Comprehensive Analysis of Non-coding RNA Profiles of Exosome-Like Vesicles From the Protoscoleces and Hydatid Cyst Fluid of *Echinococcus granulosus*. *Front Cell*

37. XueQi T. Screening of differentially expressed long non-coding RNA by the recombinant antigen P29 induced immune protection after *Echinococcus granulosus* infected host. *Ningxia Medical University* 2017.
38. Li D, Du X, Zhu M, Yang S,Zhao W. MiR-374b-5p Regulates T Cell Differentiation and Is Associated with rEg.P29 Immunity. *BIOMED RES INT* 2020; **2020**: 8024763.
39. Baz A, Carol H,Fernández V, *et al.* *Echinococcus granulosus*: Induction of T-independent antibody response against protoscolex glycoconjugates in early experimental infection. *EXP PARASITOL* 2008; **119**: 460-466.
40. Rahimi HR, Sarkari B, Mohammadzadeh T,Sadjjadi SM. Immune responses to antigens of in vitro reared *Echinococcus granulosus* adult worms in Balb/c mice. *Iranian journal of immunology : IJI* 2011; **8**: 236.
41. West KA,Lagos D. Long Non-Coding RNA Function in CD4(+) T Cells: What We Know and What Next? *Noncoding RNA* 2019; **5**: 43.
42. Zhang F, Liu G, Wei C, Gao C,Hao J. Linc-MAF-4 regulates Th1/Th 2 differentiation and is associated with the pathogenesis of multiple sclerosis by targeting MAF. *The FASEB Journal* 2016; **31**: 519-525.
43. Schopf LR, Hoffmann KF, Cheever AW, Urban JJ,Wynn TA. IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. *J IMMUNOL* 2002; **168**: 2383-2392.

Figures

Figure 1

Microarray analysis reveals significant down-regulation of lncRNA028466 transcripts in spleen lymphocytes of mice immunized with rEg.P29 antigen. (a) rEg.P29 antigen was purified and detected by Weston blot. (b) Validation of lncRNA028466 expression levels by qRT-PCR. Data were shown as mean $\pm$ SD and represented three separate experiments.***:P<0.001.

Figure 2

The expression of IncRNA028466 was decreased in CD4+T of spleens from mice immunized with rEg.P29 antigen. Following boost immunization, isolated CD4+T, CD8+T and B cells and the percentage of the three group cells were shown respectively in the control (a) and immune group (b) by flow cytometry. The expression of IncRNA028466 in CD4+T (c), CD8+T (d) and B (e) cells was measured by qRT-PCR. Results were shown as mean $\pm$ SD and represented three separate experiments. **: $P \leq 0.01$, ns: no significance.

Figure 3

Overexpression of IncRNA028466 biases naive CD4+T cell differentiation toward the Th2 phenotype. (a) Validation of the transfection efficiency of IncRNA028466 overexpression lentivirus vector by qRT-PCR. The mRNA expressions of cytokines were measured by qRT-PCR (b-e) and the supernatants were detected by ELISA (f-i). Vector represented empty vector, pCDH-028466 represented IncRNA028466 overexpression vector. Data were expressed as mean $\pm$ SD and represented three separate experiments. *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

Figure 4

Overexpression of lncRNA028466 biases naive CD4⁺T cell differentiation toward the Th2 phenotype. The differentiation of the transfected naive CD4⁺T cells were measured by FACS. The scattered dots in the box represented the expression of IFN- γ (a) and IL-2 (b) producing Th1 cells, and IL-4 (c) and IL-10 (d) producing Th2 cells. The data came from a single experiment and represented three separate experiments. *: $P \leq 0.05$, **: $P \leq 0.01$.

Figure 5

Down-regulation of lncRNA028466 promotes naive CD4+T cell differentiation toward the Th1 phenotype. (a) The interference efficiency of siRNAs was tested by qRT-PCR. The mRNA expressions of cytokines in the transfected naive CD4+T cells were tested by qRT-PCR (b-e) and the supernatants were detected by ELISA (f-i). Negative represented the control group, siRNA1 represented lncRNA028466 knockdown group. Data were expressed as mean $\pm$ SD and represented three separate experiments. *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$, ns: no significance.

Figure 6

Down-regulation of lncRNA028466 promotes naive CD4⁺T cell differentiation toward the Th1 phenotype. The differentiation of the transfected naive CD4⁺T cells were measured by FACS. The scattered dots in the box represented the expression of IFN- γ (a) and IL-2 (b) producing Th1 cells, and IL-4 (c) and IL-10 (d) producing Th2 cells. The data came from a single experiment and represented three separate experiments. *: $P \leq 0.05$, **: $P \leq 0.01$, ns: no significance.