EPIDEMIOLOGY AND HEALTH,
ISSN / eISSN 2092-7193
Publisher KOREAN SOC EPIDEMIOLOGY,
DEPT SOCIAL & PREVENTIVE MEDICINE,
Korean Red cross Hospital ,
SUNGKYUNKWAN UNIV. SCH MEDICINE,
Volume 26-Issue 1-(2026)
DOI:https://doi-xx.org/1050/17697001557730

MicroRNA-15a-5p Regulates Th1/17 Proliferation in Ovarian Cancer Ascites by Targeting CDK4

Lin-Jiao Jia[1], Xin-Long Hu[2], Su-Feng Fan[3], Yu-Jie Xu[3], Jin-Long Hu[3,*]

[1]Department of Breast Surgery, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou 450003, Henan Province, China

[2]Department of Medical Imaging, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou 450003, Henan Province, China

[3]Department of Oncology, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou 450003, Henan Province, China

[*]Correspondence to: Jin-Long Hu, E-mail: longlonghjl0305@zzu.edu.cn; Tel: +86-371-65580722

Acknowledgment: This study was supported by the Henan Provincial Department of Science and Technology, No. 212102310047 and the Henan Provincial Health Commission, No. SBGJ202003005.

Conflict of interests： No conflict of interest exits in the submission of this manuscript, and all authors have read and approved the submitted manuscript.

ABSTRACT

Advanced ovarian cancer is accompanied by malignant ascites in 80 to 90% of patients, which contain many immune cells, such as $CD4^+$ T. We aim to investigate the regulatory pathway through which Th1/Th17 cells affect the progression of ovarian cancer ascites. We examined the correlation between the frequency regarding $CD4^+$ T cells & inflammatory lymphocytes within mice ovarian cancer ascites and their survival time. Moreover, we screened the mRNA and miRNA expression profiles of ovarian cancer ascites and peripheral blood $CD4^+$T, and CDK4 was found to be down-regulated in ascites. The interaction among miR-15a-5p and CDK4 was observed using dual-luciferase reporter assays combined with CHIP-qPCR. Our study revealed that miR-15a-5p could influence the Th1/17 cell proliferation in ovarian cancer ascites through targeting CDK4. This study furthers the understanding behind the mechanism of host immunity in ovarian cancer ascites and provides new insight for the clinical diagnosis and treatment of peritoneal effusion of ovarian cancer.

Keywords

Ovarian cancer ascites, MiR-15a-5p, CDK4, Th1/17, Proliferation

INTRODUCTION

is within the leading prevalent cancers of the female genital system and exhibits the maximum fatality rate among every obstetric cancers. Due to the insidious onset and the special anatomical position, effective methods for early diagnosis are lacking. As a result, the majority of patients present with advanced disease at the time of therapeutic intervention. For cases in which the ovarian cancer lesion cannot be removed completely, the patient will relapse within 5 years, even if they received chemotherapy [1].

Advanced ovarian cancer is accompanied by malignant ascites in 80 to 90% of patients. Malignant ascites are predictive of poor quality of life and short survival time. In recent years, immune response within that emergence along with progression regarding pelvic tumor has existed widely studied. Recent studies have suggested that peritoneal metastasis of ovarian cancer cells, peritoneal lymphatic obstruction, and vascular permeability change are important causes of peritoneal fluid accumulation in ovarian cancer. However, these findings cannot fully explain the mechanism behind the emergence and progression of ovarian malignancy ascites.

The ascites contain many immune cells, such as lymphocytes, macrophages, and monocytes, which can be used to evaluate the immune status of ovarian cancer patients through a liquid biopsy. Ovarian tumors are immunogenic and can stimulate the body's immune system to create various responses [2]. T lymphocytes are specific immune cells in the immune system that directly or indirectly inhibit the growth of tumor cells [3]. Studies have also reported that the percentage of $CD^{4+}CD25^{+}$Foxp3+ modulatory T-like cell populations withinindividuals with ovary neoplasm is associated with the prognosis of patients [4]. Compared with normal human peripheral blood, the $CD3^{+}CD4^{+}$T cells in ascites of ovarian cancer patients were reduced in naive subsets and increased in effector memory subsets [5].

It was reported that patients with a low percentage of $CD3^{+}CD4^{+}$T cells in abdominal effusion tend to have improved clinical outcomes [6]. Therefore, further study of $CD4^{+}$T cell subsets in the ovarian cancer ascites is beneficial for optimizing

the diagnosis and treatment of ovarian cancer patients. $CD4^+$T lymphocytes can be differentiated into four functional subsets under different cytokines: Th1, Th2, Treg, and Th17 cells [7]. Th1 cells can enhance the host immunity to the infection of pathogenic microorganisms, especially viruses and intracellular pathogens. Th17 cells could produce IL-17, which is associated with the emergence and progression of multiple immune reactions. Cytokines secreted by Th1 cell subsets, especially interleukin 2 and IFN-γ, can activate and promote the functional role of $CD8^+$ T and NK cells [3]. Meanwhile, the researchers found that Th17 cells played a role in promoting anti-tumor immunity, and IFN-γ and IL-17 produced by Th17 cells could promote $CD8^+$ T cell migrate to the tumor microenvironment in a dose-dependent manner. Therefore, adopting Th17 cells into the tumor microenvironment or promoting the transformation of original T cells into Th17 cells may also benefit patients with advanced ovarian cancer.

In an analysis of 201 ovarian cancer patients, tumor-infiltrating Th1 cells exhibited diverse effector phenotypes and demonstrated a positive association with effector T cells, while being inversely linked to regulatory T cells in the tumor microenvironment. Additionally, tumor-associated macrophages facilitated Th17 cell differentiation via IL-1β, whereas regulatory T cells exerted a suppressive effect on Th17 activity [8]. Moreover, in malignant ascites caused by liver cancer, Th17 cells and IL-17 were significantly elevated compared with the blood of healthy individuals [9]. However, the role of Th1/17 cells in ovarian cancer ascites needs to be studied further.

Cyclin-dependent kinase 4 (CDK4) plays an essential role in driving the G1-to-S phase progression and is crucial for the development, proliferation, and maintenance of several malignancies [10]. However, the study of CDK4 in ovarian cancer ascites is lacking. Studies investigating ovarian cancer show that the abnormal manifestation of non-coding RNA is associated with the occurrence and development of ovarian cancer. MiRNAs can function as tumor suppressor genes or oncogenes in tumorigenesis. Recent Research indicate that the reasons for abnormal expression of miRNA in tumors are chromosome rearrangement [11], genome copy number change, epigenetic

modification [12], defects of miRNA production pathway [13], and regulation of transcription factors [14]. MiRNA is also believed to be related to the prognosis of ovarian cancer patients and can be used as a predictive target for early diagnosis of ovarian cancer [15]. Therefore, further research on the regulation of CDK4 and miRNA expression in ovarian cancer ascites and there influence in Th1/Th17 cells will continue to clarify the emergence as well as progression of ovary neoplasm ascites.

In this analysis, we examined the role of CDK4 in Th1 and Th17 cells in ovarian cancer ascites, and the regulatory role of miR-15a-5p on CDK4. The mechanism behind ovarian cancer ascites was investigated from the perspective of host immunity with the aim of adding to the understanding of the pathogenesis of ovarian cancer ascites.

MATERIALS AND METHODS

Cell culture

Commercial cell cultures 293T and ID8 were acquired from ATCC. These cultures were cultivated in a moist chamber containing 5% CO_2 under 37° C in Eagle's Modified Dulbecco Medium (Gibco), fortified via 10% young bovine serum (YBS; Thermo Fisher). Original cells of mouse-derived ascites were cultured in DMEM Medium (Thermo Fisher), enriched with 10% FCS.

Mouse model and sample processing

C57BL/6N wt mice were purchased from SiPeiFu Biotechnology Co., Ltd. (Beijing, China). Mouse models of ovarian cancer ascites were induced by an intraperitoneal injection of ID8 cells (1.0×10^5 each), and the baseline group was administered with identical volume of normal saline. Seven weeks after the injection, mice were euthanized by CO_2 asphyxiation, then the malignant ascites from model group and abdominal lavage fluid from control group and blood samples were collected from each mouse.

Mononuclear cell isolation

Lymphocyte Separation Medium (LSM) was purchased from MP Biomedicals. We loaded 4.5 ml LSM into 15 ml centrifuge tubes. Approximately 6 ml of diluted blood or ascites were decanted directly from the ethylenediaminetetraacetic acid tube into

each Lymphoprep tube and processed in a centrifuge at 500 units for 30 minutes at 20° C. The circulating plasma lymphoid cells (PBMC) enriched fraction was transferred, and red cell lysis was performed.

Flow cytometry

For cytokine detection, cellular units were activated by phorbol ester derivative (Sigma-Aldrich), ionomycin (Enzo Life Sciences), GolgiStop, and GolgiPlug (BD Biosciences) for 6 hours before staining. The mice cells were first stained extracellularly with antibodies against CD45, CD3, CD8, CD4 (BD Biosciences), and CD19 (R&D Systems), incubated at 4°C for 15 minutes. Then cells were fixed and permeabilized with Fixation/Permeabilization solution (eBioscience) for 25 minutes, and finally stained intracellularly with anti-IL-17 (R&D Systems), anti-IFN-γ (eBioscience) for 20 minutes.

$CD4^+$T cell isolation

CD^{4+}T cells were extracted using the method mouse $CD4^+$T cell isolation kit (Miltenyi Biotec) based on the maker's guidelines. The PBMCs were mixed with magnetic beads and incubated at 4°C in a buffer for 15 minutes, and then the cell suspension was added to the separation column. After washing the column with 3 ml buffer, a pipette with 5 mL of solution was added into the matrix. Finally, The chemically tagged CD^{4+} lymphocytes were flushing out through forcefully depressing the piston toward the cylinder.

Real-time polymerase chain reaction

First, whole genetic material was isolated from organisms using RNeasy Kit (Tiangen Biotech, Beijing, China). Second, cDNA was transcribed using a PrimeScript RReverse Transcription reagent Set with genomic DNA Removal Agent (manufactured by Takara, Japan). Both the RNA extraction and the cDNA reverse transcription were carried out in line with the corresponding manufacturer’s instruction. Live-action DNA synthesis technique (DST) was executed using the SYBR Premix Ex TaqII (Takara, Japan), using the LightCycler 480 System (Roche). The primers were as follows: for GAPDH,

forward 5'-GAA GGT GAA GGT CGG AGT C-3' and reverse 5'-GAA GAT GGT GAT GGG ATT TC-3'; for CDK4, forward 5'-GTG GAC ATG TGG AGT GTT GG-3', and reverse 5'-GGA TAC ATC TCG AGG CCA GT-3'. Comparative assessment of genetic activity was conducted with the $2^{-\Delta\Delta CT}$ method.

Western blot

Total protein was extracted using a radioimmunoprecipitation assay lysate solution and measured using the Thermo Scientific BCA Protein Assay Kit (Thermo Scientific). Western blot was performed with primary antibodies against CDK4 (1:1000, Cell Signaling Technology) and GAPDH (1:1000, Cell Signaling Technology). The secondary antibody was Goat anti-Rabbit IgG conjugated with HRP. Protein bands were imaged using SuperSignal West Pico PLUS (Thermo Scientific).

Chromatin immunoprecipitation assay

The cellular units were preserved using a 1% formalin solution for 10 minutes. Next, the nuclear protein was extracted by ultrasonic fragmentation. Chromatin protein was incubated with the CDK4 antibody or the Goat anti-Rabbit IgG (Cell Signaling Technology) and stabilized through the night at 4° C. The antigen-antibody complexes were purified on Protein A-coated agarose particles using a chromatin immunoprecipitation Assay Kit (Thermo Scientific). The nucleoprotein structures were purified from the resin, and DNA bridging was later disrupted.

Dual-luciferase reporter assay

Wild type and mutant plasmids of CDK4 mRNA were constructed to perform dual-luciferase reporter assay. 293T cells were transfected with luciferase reporter gene plasmid, lacZ expression plasmid and hsa-miR-15a-5p mimic or inhibitor. After 24 h after gene delivery, cellular units were disrupted in a non-active lysis solution, and photinus and sea pansy luciferase activity levels were assessed via the Dual-Reporter Testing Set (Promega) in accordance with the producer's guidelines.

Cell proliferation assay

Cells were reversely transfected with indicated siRNAs or control using Lipofectamine 2000 (Thermo Scientific) and seeded at 5 x 10^3 in 96-well plates (n = 5 / condition). At the indicated time points, CCK8 assays were performed with Cell Counting Kit-8 (APE x BIO), following the manufacturer's guidelines. After incubation at 37°C for 1h, OD450 was measured with a microplate reader.

Statistics

The distribution uniformity was assessed using an Anderson-Darling procedure. A Student's t-assessment was used to compare the groups with data conforming to the normal distribution, expressed as Mean ± SD. The distribution-free Wilcoxon sum-rank method (commonly referred to as the Mann-Whitney U procedure) was used to compare the groups with a non-normal distribution of data as the median (interquartile distance, IQR). For comparison among multiple groups, one-way or two-way ANOVAs were used with the Bonferroni test for pair-to-group comparison. The Kruskal-Wallis analysis was applied to the non-normal distribution data, with Dunn's method for post hoc analysis. Pearson's chi-square test (χ2 test) served as to analyze the discrete variables. A Significance threshold of 0.05 was deemed statistically meaningful.

RESULTS

The ratio of Th1/17 cells was associated with the survival of mice with ovarian cancer ascites

We constructed a mouse ovarian cancer ascites model with ID8 cells in C57BL/6N wt mouse species and analyzed the correlation between Th1 and Th17 in PBMC and ascites of ovarian cancer and the survival time of mice by flow cytometry. The results showed a nonsignificant negative association between the share of Th1 cells and the survival of mice with ovarian cancer peritoneal fluid accumulation ($r = -0.3906$, $P = 0.089$), while the proportion of Th17 cells showed a significant positive correlation with the survival of mice with ovarian cancer peritoneal fluid accumulation ($r = 0.7412$, $P <$

0.01; Figure 1). This suggests that Th17 cells may play an important role in the occurrence and progression of ovarian malignancy ascites.

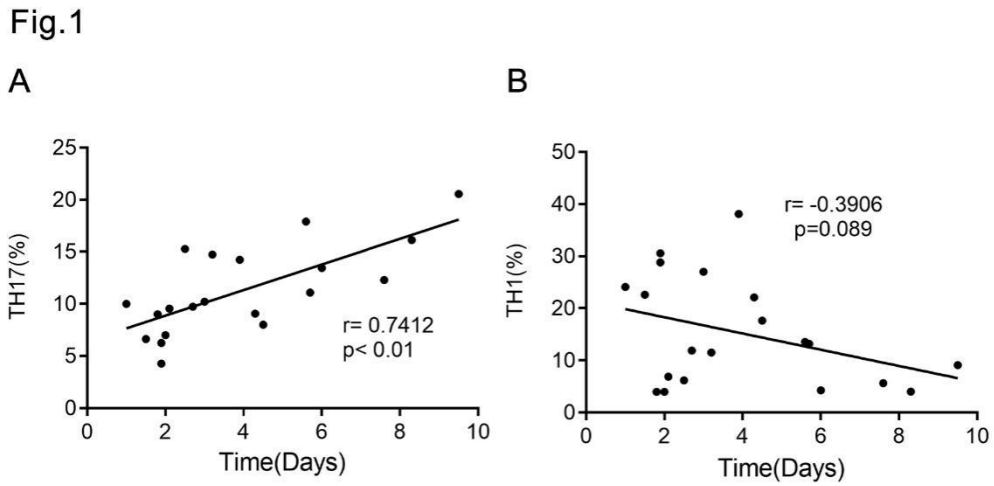

Figure 1. A Correlation between Th17 cellular units within peritoneal effusion from rodent ovary neoplasm and mouse survival. **B** Correlation between Th1 cellular elements within peritoneal effusion of murine ovary neoplasm and mouse survival.

CDK4 was highly expressed in CD4$^+$T cells in ovarian cancer ascites

The abdominal effusion and PBMC of mouse ovarian cancer ascites were collected, CD4$^+$T lymphocytes were sorted, and total RNA was obtained. To compare CDK4 gene mRNA levels in abdominal effusion and peripheral blood CD4$^+$T lymphocytes between ovarian cancer mice and the control group, An reverse transcription-quantifying genetic material replication technique (RT-qPCR) was utilized for quantifying RNA. The CDK4genetic element was detected strongly activated within helper T lymphocytes in the ovarian cancer ascites compared to the control group (Figure 2), which suggest that CDK4 may serve a crucial function in the advancement of ovarian malignancy ascites.

Fig.2

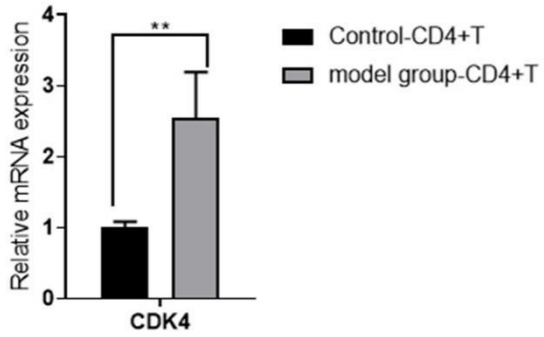

Figure 2. Comparison of RT-qPCR of CDK4 in ovarian cancer ascites and peripheral blood $CD4^{+}T$ lymphocytes between control and model group. * $*P < 0.01$.

hsa-miR-15a-5p restrained the CDK4 expression

Hsa-miR-15a-5p mimic and inhibitor were transfected to detect CDK4 mRNA levels. After transfection with the microRNA mimic, the CDK4 mRNA level was downregulated compared with negative control. When cells were transfected with the inhibitor, the CDK4 transcription level was upregulated. Similar to the mRNA changes, after transfection with hsa-miR-15a-5p mimic, the protein level of CDK4 was downregulated compare with negative control. While after transfecting the microRNA inhibitor,, the protein level of CDK4 was upregulated (Figure 3). These findings imply that hsa-miR-15a-5p might modulate the activity rate of CDK4.

Fig.3

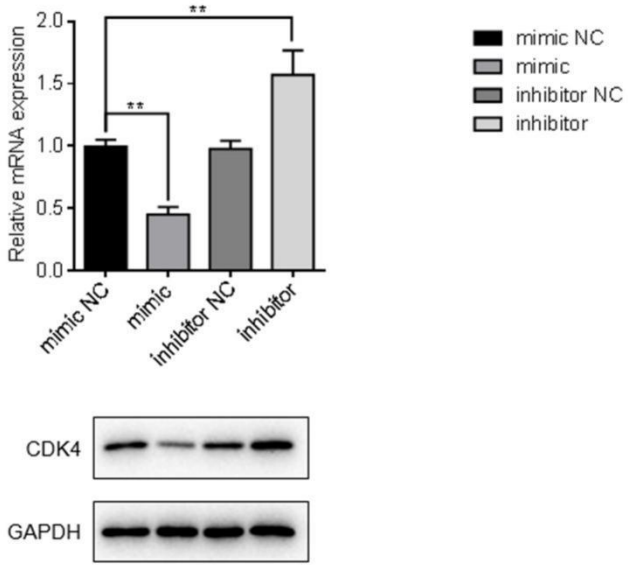

Figure 3. RT-qPCR and western blot of CDK4 after miR-15a-5p mimic or inhibitor interference compared to NC. ** $P < 0.01$.

hsa-miR-15a-5p targeted the 3'UTR of CDK4 gene

We constructed CDK4 luciferase plasmid and transfected hsa-miR-15a-5p mimic and inhibitor into the 293T cell lines. Next, the luciferase activity was detected (Figure 4A). Introduction via the hsa-miR-15a-5p analog led to a reduction in CDK4 expression, whereas introduction of its inhibitor resulted in elevated CDK4 levels. The mutant plasmid of CDK4 3'UTR was not influenced by hsa-miR-15a-5p mimics or inhibitors. RNA pull-down assay was used to identify whether hsa-miR-15a-5p could target CDK4. The findings indicated that miRNA-15a-5p may bind to CDK4 mRNA 3'UTR (Figure 4B). These findings suggest that Non-coding RNA fragment could interact with the 3' untranslated region of CDK4 gene and regulate the gene's expression.

Fig.4

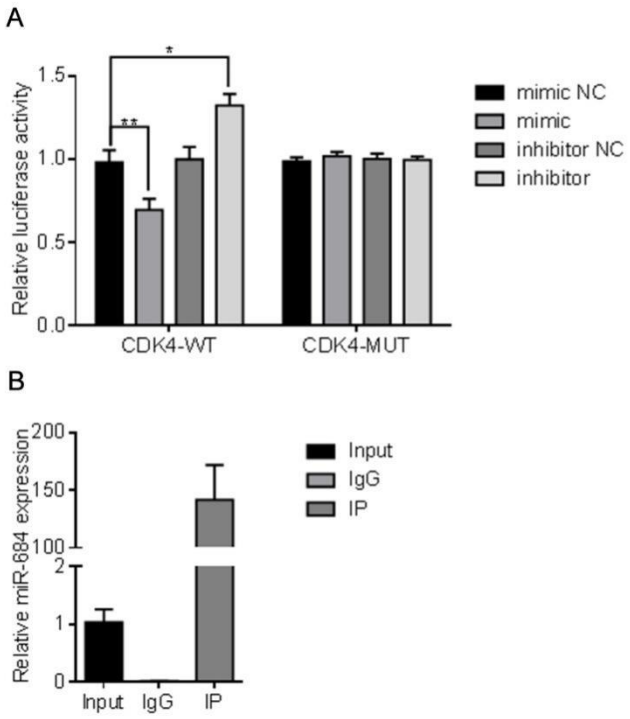

Figure 4. A The relative luciferase activity with miR-15a-5p mimic or inhibitor in CDK4-WT and -MUT groups in 293T cell line. **B** Nucleic acid affinity purification test was conducted to confirm the interaction between miR-15a-5p and CDK4 mRNA, qPCR of CDK4 mRNA pulldown assay. * $P < 0.05$. ** $P < 0.01$.

CDK4 affected the proliferation of Th1/17 cells

We further screened the target cells regulated by CDK4-mediated differential expression of target genes. Peritoneal effusion and peripheral blood lymphocytes of the mice was isolated, and $CD4^+$T, $CD8^+$T and B cells were separated by fluorescence-activated cell sorting. Complete RNA was isolated using a reagent and transcribed in reverse into cDNA. The expression of the above differential genes was verified in these cells and the target cells of related genes were identified (Figure 5A). We then examined the influence of CDK4 protein on CD4+ lymphocytes subsets. We isolated Th1 and Th17 cells from the mouse ovarian cancer ascites and conducted CDK4 gene knockdown. Expansion of T helper 1 and T helper 17 cell populations was assessed

using CCK-8 assays (Figure 5B). These experiments revealed that CDK4 enhances Th17 cell expansion while suppressing Th1 cell growth in ovarian cancer ascites.

Fig.5

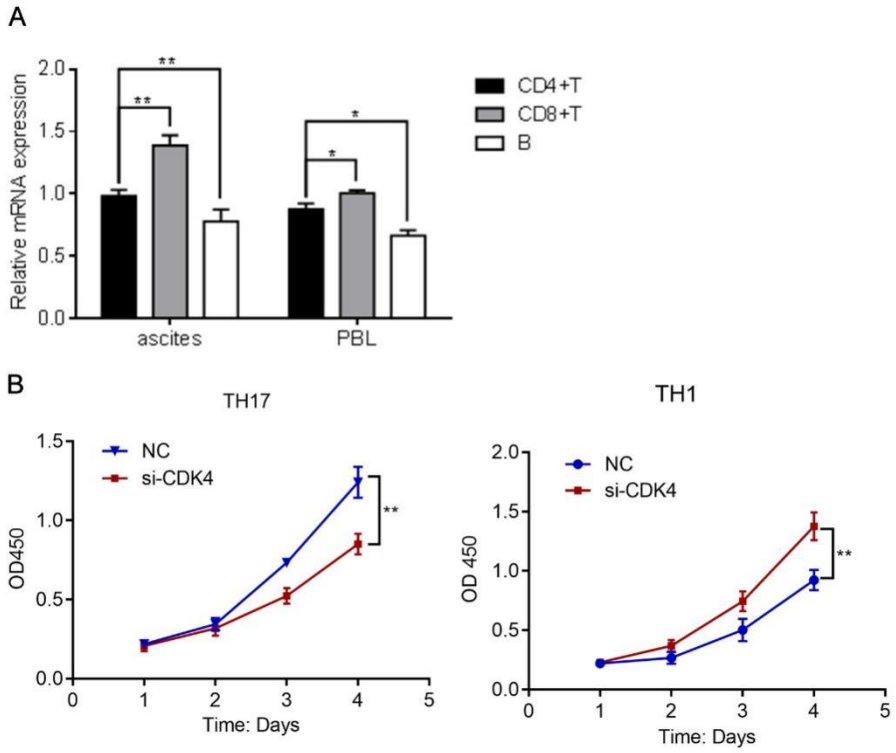

Figure 5A. Comparison of RT-qPCR of mRNA in $CD4^+T$, $CD8^+T$ and B lymphocytes in ovarian cancer ascites and peripheral blood (PBL). **5B.** The cells proliferation difference between NC and si-CDK4 on OD450 over days in Th17 and Th1 cells. * $P < 0.05$.** $P < 0.01$.

DISSCUSSION

We investigated the correlation between the proportion of Th1/17 tissues and the lifespan of rodents afflicted with gynecological tumors peritoneal effusion. It was found that the ratio of Th1 lymphocytes inversely related to the survival time of mice with ovarian cancer ascites, while the proportion of Th17 cells showed a positive correlation. This suggests that Th1 cells and Th17 cells may play vital roles in the emergence and progression of gynecological malignancy ascites. We further applied RNA sequencing to investigate the differences in mRNA and miRNA expression profiles of ovarian

cancer ascites and peripheral blood $CD4^{+}$T lymphocytes. We next analyzed and clarified the modulatory interaction between hsa-miR-15a-5p and the CDK4 gene, as well as ta modulatory process of hsa-miR-15A-5P within the mouse ovarian cancer abdominal fluid accumulation model. To clarify the regulatory mechanism of miRNA CDK4, we investigated its impact on the CDK4–Th1/Th17 axis in malignant ascites from a murine ovarian cancer model, assessing its correlation with ascites development and overall survival.

CDK4 is a critical mediator in the cell transition to the S phase and is import for the initiation, growth and survival of many cancer types [10]. The oncogenic potential of CDKs is further evidenced in murine models. For example, targeted overexpression of CDK1 in the mammary glands of transgenic mice resulted in mammary tumor formation [16]. In addition, overexpression of cyclin D2, D3, or CDK4 can also lead to tumor formation [17]. On the contrary, CDK4 ablation reduces tumor sensitivity. For example, CDK4 deficient mice or kinase inactivated CDK4 knock-in mice were resistant to HER2-driven breast cancer[18-21]. In the rodent system of large-cell pulmonary carcinoma (NSCLC) induced through KRAS, acute ablation of CDK4 depressed tumor cell proliferation and triggered tumor cell senescence [22]. CDK4 inhibition promotes immune infiltration in ovarian cancer, enlarges T cell effector populations and promotes memory T cell formation [23, 24].

Study on miR-15a-5p demonstrates its role in suppressing colon cancer progression through regulation of the G1/S-specific cyclin-D1 (CCND1) gene [25]. Furthermore, plasma exosomal miR-15a-5p shows significant potential as a diagnostic biomarker for early-stage endometrial cancer [26]. This molecule also impedes lung cancer cell metastasis by disrupting lipid metabolism through inhibition of acetyl-CoA metabolic activity and chromatin acetylation modification [27]. These studies demonstrate that miR-15a-5p plays an important role in several cancer types.

Nevertheless, the roles of miR-15a-5p within ovarian cancer and associated ascites remain poorly characterized. In this study, we show that miR-15a-5p directly interacts with the 3′-UTR of CDK4 and downregulates its expression, which enhances Th1 cell

expansion while suppressing Th17 cell proliferation in ovarian cancer ascites. The involvement of Th1 and Th17 cells in ascites formation and progression will be the subject of future investigations.

In conclusion, this study provides a new potential target and a new idea for clinical diagnosis and treatment of peritoneal effusion of ovarian cancer.

FUNDING: This study was supported by the Henan Provincial Department of Science and Technology, No. 212102310047 and the Henan Provincial Health Commission, No. SBGJ202003005.

CONFLICT OF INTEREST：No conflict of interest exits in the submission of this manuscript, and all authors have read and approved the submitted manuscript.

REFERENCE

[1] Koole SN, Van Lieshout C, Van Driel WJ et al.Cost Effectiveness of Interval Cytoreductive Surgery With Hyperthermic Intraperitoneal Chemotherapy in Stage III Ovarian Cancer on the Basis of a Randomized Phase III Trial. J Clin Oncol. 2019;37(23):2041-+. https://doi.org/10.1200/Jco.19.00594.

[2] Funingana I-G, Bedia JS, Huang Y-W et al.Multiparameter single-cell proteomic technologies give new insights into the biology of ovarian tumors. Seminars in Immunopathology. 2023;(1863-2300 (Electronic)).https://doi.org/10.1007/s00281-022-00979-9.

[3] Knutson KL, Disis ML.Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. Cancer Immunol Immun. 2005;54(8):721-8.https://doi.org/10.1007/s00262-004-0653-2.

[4] Moser M, Preston CC, Maurer MJ et al.The Ratios of CD8+ T Cells to CD4+CD25+ FOXP3+ and FOXP3- T Cells Correlate with Poor Clinical Outcome in Human Serous Ovarian Cancer. PLoS ONE. 2013;8(11).https://doi.org/10.1371/journal.pone.0080063.

[5] Lieber S, Reinartz S, Raifer H et al.Prognosis of ovarian cancer is associated with effector memory CD8+ T cell accumulation in ascites, CXCL9 levels and activation-triggered signal transduction in T cells. Oncoimmunology. 2018;7(5).https://doi.org/10.1080/2162402x.2018.1424672.

[6] Wefers C, Duiveman-De Boer T, Yigit R et al.Survival of Ovarian Cancer Patients Is Independent of the Presence of DC and T Cell Subsets in Ascites. Front Immunol. 2019;9(1664-3224 (Electronic)).https://doi.org/10.3389/fimmu.2018.03156.

[7] Al-Kufaidy R, Vazquez-Tello A, Bahammam AS, Al-Muhsen S, Hamid Q, Halwani R.IL-17 enhances the migration of B cells during asthma by inducing CXCL13 chemokine production in structural lung cells. J Allergy Clin Immun. 2017;139(2):696-+.https://doi.org/10.1016/j.jaci.2016.07.037.

[8] Kryczek I, Banerjee M, Cheng P et al.Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor

environments. Blood. 2009;114(6):1141-9. https://doi.org/10.1182/blood-2009-03-208249.

[9] Yang XW, Jiang HX, Lei R, Lu WS, Tan SH, Qin SY. Recruitment and significance of Th22 cells and Th17 cells in malignant ascites. Oncol Lett. 2018;16(4):5389-97. https://doi.org/10.3892/ol.2018.9316.

[10] Goel S, Bergholz JS, Zhao JJ. Targeting CDK4 and CDK6 in cancer. Nature Reviews Cancer. 2022;22(6):356-72. https://doi.org/10.1038/s41568-022-00456-3.

[11] Jiang W, Zhang W, Wu L et al. MicroRNA-Related Polymorphisms in PI3K/Akt/mTOR Pathway Genes Are Predictive of Limited-Disease Small Cell Lung Cancer Treatment Outcomes. Biomed Res Int. 2017;2017(2314-6141 (Electronic)):1-10. https://doi.org/10.1155/2017/6501385.

[12] Jiang W, Bi N, Zhang WJ et al. MicroRNA-related polymorphisms in apoptosis pathway genes are predictive of clinical outcome in patients with limited disease small cell lung cancer. Oncotarget. 2016;7(16):22632-8. https://doi.org/10.18632/oncotarget.8134.

[13] Lee JS, Ahn Y-H, Won HS, Sun DS, Kim YH, Ko YH. Prognostic Role of the MicroRNA-200 Family in Various Carcinomas: A Systematic Review and Meta-Analysis. Biomed Res Int. 2017;2017(2314-6141 (Electronic)):1-11. https://doi.org/10.1155/2017/1928021.

[14] Wallaert A, Durinck K, Taghon T, Van Vlierberghe P, Speleman F. T-ALL and thymocytes: a message of noncoding RNAs. J Hematol Oncol. 2017;10(1). https://doi.org/10.1186/s13045-017-0432-0.

[15] Banno K, Yanokura M, Iida M et al. Application of MicroRNA in Diagnosis and Treatment of Ovarian Cancer. Biomed Res Int. 2014;2014(2314-6141 (Electronic)):1-6. https://doi.org/10.1155/2014/232817.

[16] Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A, Schmidt EV. Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. Nature. 1994;369(6482):669-71. https://doi.org/10.1038/369669a0.

[17] Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. Nature Reviews Cancer. 2017;17(2):93-

115. https://doi.org/10.1038/nrc.2016.138.

[18] Landis MW, Pawlyk BS, Li T, Sicinski P, Hinds PW. Cyclin D1-dependent kinase activity in murine development and mammary tumorigenesis. Cancer Cell. 2006;9(1):13-22. https://doi.org/10.1016/j.ccr.2005.12.019.

[19] Reddy HKDL, Mettus RV, Rane SG, Gran A X, Litvin J, Reddy EP. Cyclin-Dependent Kinase 4 Expression Is Essential for Neu-Induced Breast Tumorigenesis. Cancer Research. 2005;65(22):10174-8. https://doi.org/10.1158/0008-5472.Can-05-2639.

[20] Yu Q, Sicinska E, Geng Y et al. Requirement for CDK4 kinase function in breast cancer. Cancer Cell. 2006;9(1):23-32. https://doi.org/10.1016/j.ccr.2005.12.012.

[21] Yu Q, Geng Y, Sicinski P. Specific protection against breast cancers by cyclin D1 ablation. Nature. 2001;411(6841):1017-21. https://doi.org/10.1038/35082500.

[22] Puyol M, Martín A, Dubus P et al. A Synthetic Lethal Interaction between K-Ras Oncogenes and Cdk4 Unveils a Therapeutic Strategy for Non-small Cell Lung Carcinoma. Cancer Cell. 2010;18(1):63-73. https://doi.org/10.1016/j.ccr.2010.05.025.

[23] Zhang Q-F, Li J, Jiang K et al. CDK4/6 inhibition promotes immune infiltration in ovarian cancer and synergizes with PD-1 blockade in a B cell-dependent manner. Theranostics. 2020;10(23):10619-33. https://doi.org/10.7150/thno.44871.

[24] Ali LR, Garrido-Castro AC, Lenehan PJ et al. PD-1 blockade and CDK4/6 inhibition augment nonoverlapping features of T cell activation in cancer. Journal of Experimental Medicine. 2023;220(4). https://doi.org/10.1084/jem.20220729.

[25] Li Z, Zhu Z, Wang Y et al. hsa-miR-15a-5p inhibits colon cell carcinoma via targeting CCND1. Molecular Medicine Reports. 2021;24(4). https://doi.org/10.3892/mmr.2021.12375.

[26] Zhou L, Wang W, Wang F et al. Plasma-derived exosomal miR-15a-5p as a promising diagnostic biomarker for early detection of endometrial carcinoma. Molecular Cancer. 2021;20(1). https://doi.org/10.1186/s12943-021-01352-4.

[27] Ni Y, Yang Y, Ran J et al. miR-15a-5p inhibits metastasis and lipid metabolism by suppressing histone acetylation in lung cancer. Free Radical Biology and Medicine. 2020;161:150-62. https://doi.org/10.1016/j.freeradbiomed.2020.10.009.