Original Article

Iranian Journal of Immunology

https://iji.sums.ac.ir

CD39 Expression in Peripheral T Cells is Associated with Clinicopathological Characteristics in Patients with Cervical Cancer

Kuo Zhao[1,2], Dongmei Han[3,4], Lu Tang[5], Hao Jin[3,4*]

[1]Oncology Department, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China; [2]Oncology Department, Tianjin Cancer Hospital Airport Hospital, Tianjin, China; [3]Center for Precision Cancer Medicine and Translational Research, Tianjin Cancer Hospital Airport Hospital, Tianjin, China; [4]Center for Precision Cancer Medicine and Translational Research, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China; [5]Division of Rheumatology, Tianjin First Center Hospital, Tianjin, China

ABSTRACT

Background: CD39 is an inhibitory checkpoint exerting rate-limiting effects on ATP-adenosine pathway. It can be targeted to block adenosine-mediated immunosuppression.

Objective: To analyze the relationship between the CD39 expression and clinicopathological characteristics including FIGO stage, lymph node and distant metastasis, and to further explore its potential role in cervical cancer.

Methods: Peripheral blood was collected from 59 healthy people and 43 patients with cervical cancer. The percentage and absolute counts of CD3-positive, CD4-positive and CD8-positive T lymphocytes, CD4/CD8 ratio and the percentage of the $CD39^+$ T cells in T lymphocytes were assessed by flow cytometry, and their correlations with clinical parameters were analyzed.

Results: Absolute numbers of $CD8^+$ T lymphocytes, CD4/CD8 ratios, and the percentage of the $CD39^+$ T cells were linked with FIGO stage, lymph node metastasis, and distant metastasis. The total numbers of $CD8^+$ T lymphocytes were significantly higher in the peripheral blood of patients with cervical cancer in the early and middle stages than in the advanced stage. In addition, patients with early and middle-stage cervical cancer had considerably lower percentage of $CD4^+CD39^+$ and $CD8^+CD39^+$ T lymphocytes than those with advanced cervical cancer.

Conclusion: These results suggest that the absolute counts of $CD8^+$ T lymphocytes may be associated with the patient's prognosis and that the CD39 molecule, expressed on the surface of $CD8^+$ T cells, is also related to FIGO stage, lymph node metastasis, and distant metastasis. CD39 expression on CD8-positive T cells exhibits a negative correlation with the number of CD8-positive T lymphocytes.

Keywords: CD39 Antigen, Ectonucleoside Triphosphate Diphosphohydrolase 1, Flow Cytometry, T Lymphocyte Subsets, Uterine Cervical Neoplasms

*Corresponding author:
Hao Jin,
Tianjin Cancer Hospital Airport Hospital, No. 99, East 5th Road, Tianjin Airport Economic Zone, Tianjin, China
Email: haojin1031@126.com

Cite this article as:
Zhao K, Han D, Tang L, Jin H. CD39 Expression in Peripheral T Cells is Associated with Clinicopathological Characteristics in Patients with Cervical Cancer. *Iran J Immunol.* 2023; 20(3):276-286, doi: 10.22034/iji.2023.97037.2527.

Received: 2023-01-18
Revised: 2023-04-25
Accepted: 2023-05-03

INTRODUCTION

Due to global population growth and aging, as well as smoking, poor diet, and sedentary lifestyles, the number of cancer patients is increasing, and the global cancer burden continues to increase (1). One of the main causes of cancer-related deaths in women is cervical cancer. With an estimated 530,000 new cases, 270,000 deaths annually, and a survival rate ranging from 33% to 77%, cervical cancer is the fourth most prevalent cancer in women globally after breast, colorectal, and lung cancer (2). The etiology is not fully understood, but studies have shown that human papillomavirus (HPV) infection can be detected in 95% of cervical malignancies. According to tumor immunology, the presence and progression of cancer are highly correlated with the body`s immunological health (3, 4). T lymphocytes are the main response form of cellular immunity, playing a leading role in the body`s antitumor immunity, which can recognize and eliminate tumor cells (5, 6). Lymphocyte subset analysis by flow cytometry as a routine examination is an important indicator reflecting cellular immune function. The index CD3-positive T lymphocytes represent total T cells, including helper T lymphocytes and cytotoxic T lymphocytes. These two types of T cells are represented by CD4-positive and CD8-positive cells. Helper T cells mediate assisted immunity, while cytotoxic T cells are involved in cellular immunity. The index CD4/CD8 ratio reflects the balance between these two types of immunity. This is of great significance in tumor immune monitoring, and further provides a reference for the clinical treatment of patients (7, 8). It has been reported that adenosine, which can promote the generation of regulatory T cells and simultaneously suppress other immune cells, is an important immunosuppressive molecule in the tumor microenvironment, thereby allowing the escape of tumor cells. CD39 plays a significant part in the production of adenosine as the initiating molecule in adenosine metabolism. Ecto-nucleoside triphosphate diphosphohydrolase (NTPDase) CD39 maintains the balance of immunological response by hydrolyzing adenosine triphosphate (ATP) and adenosine diphosphate (ADP) (9). It is a recently identified immunological checkpoint mediator inhibiting the immune system`s antitumor response. Effector antitumor immunity is inhibited by adenosine, which is produced in conjunction with CD39 expression (9). Many studies have identified a variety of CD39`s esoteric roles, which are related to Tregs, Th17 cells, and Bregs (10-12). Ectonucleotidase CD39 and programmed cell death 1 receptor (PD-1) were shown to be considerably enhanced in intratumoral immune cells in non-small-cell lung cancer, according to one study (13). These findings imply that CD39 plays a crucial role in tumor immunity. This study first analyzed the proportions and absolute numbers of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes in the cervical cancer patients` peripheral blood and combined these indicators to evaluate the immune function status of the body. Then, by detecting the CD39 expression level on the surface of T lymphocytes and combining it with the clinical information of cervical cancer patients, correlation studies were carried out to explore the role of CD39, a key molecule of the adenosine metabolism pathway, affecting the immune function of cervical cancer patients.

MATERIALS AND METHODS

Study Subjects

Peripheral blood samples were obtained from 59 healthy women with an age distribution of 28-81 years and 43 cervical cancer patients, with an age range of 25-69 years, and a pathological type of squamous cell carcinoma, who were first-time admitted to Tianjin Cancer Hospital Airport Hospital, after receiving written informed consent. Prior to blood collection, none of the patients

received surgery, radiation, chemotherapy, or any other form of medical treatment.

The study was conducted in accordance with the Declaration of Helsinki (revised in 2013). The study was approved by the Ethics Committee of Tianjin Medical University Cancer Institute & Hospital in March 2016. The approval number is the same as that of the National Natural Science Foundation of China (No. 81602020). The study was also approved by the Ethics Committee of Tianjin Cancer Hospital Airport Hospital in September 2022. The approval number is JSK-2022-0062. The study was deemed exempt from institutional board approval and patient informed consent was waived, due to the retrospective nature and publicly available data source of the study.

Methods

The cells were treated with adequately diluted antibodies after the FcR had been blocked, and then the phosphate buffered saline (PBS) was applied to wash the cells. The antibodies used for surface staining in this study included anti-human CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 (Beckman Coulter) and anti-human CD39-APC (Biolegend). The acquisition was performed using Navios (Beckman Coulter). The Kaluza software (Beckman Coulter) was used for the data analysis. The flow cytometry analysis process is shown in Fig. 1.

Statistical Analysis

SPSS Statistics 21 (IBM Corporation, NY, USA) was used for all statistical analyses. As the Kolmogorov-Smirnov test showed that the numerical data had a normal distribution, the mean, and standard deviation were used to represent the data. The independent sample t-test was used to compare numerical data. When the distribution agreed with the non-normal distribution, the nonparametric t-test and Kruskal-Wallis test were performed to establish the statistical significance. The data were presented as the median and interquartile range. $p<0.05$ was considered statistically significant.

RESULTS

Cervical Cancer Patients Have a Lower Absolute Number of CD8-positive T Lymphocytes in Peripheral Blood than in the Healthy Subjects

This study includes a total of 43 instances

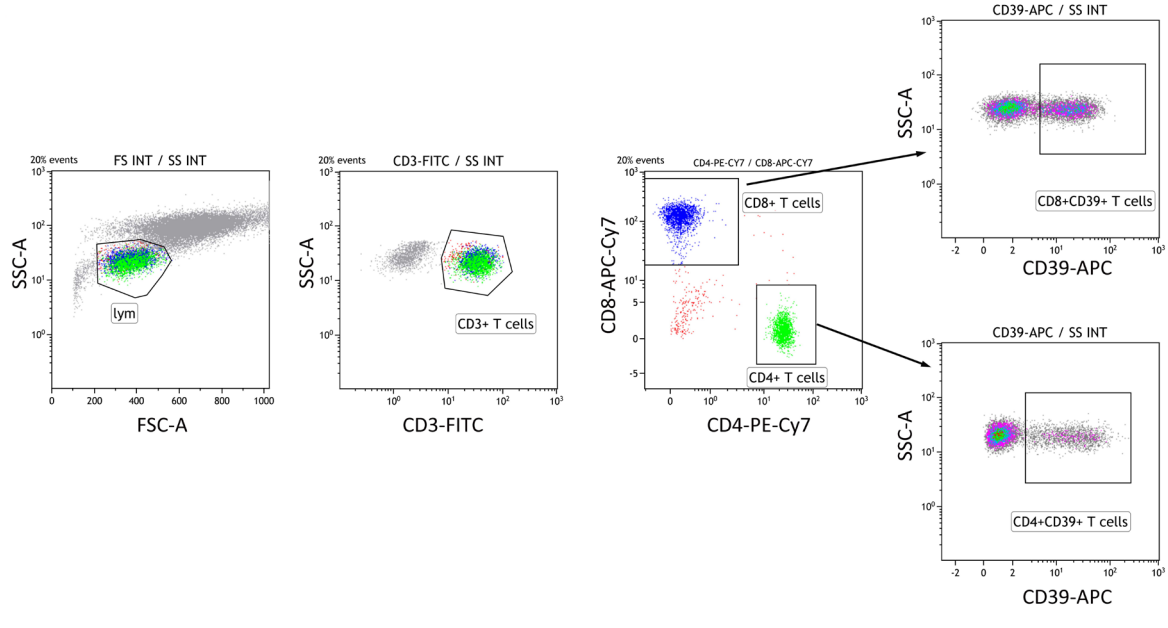

Fig. 1. Representative flow cytometry gating strategies for T lymphocytes and CD39 expression in the peripheral blood of cervical cancer patients

of cervical cancer. However, the study also collected peripheral blood samples from 59 healthy women. Data for a total of 6 parameters were collected, including the percentage and absolute counts of CD3-positive, CD4-positive, and CD8-positive T lymphocytes in the peripheral blood of patients and healthy individuals plus the CD4/CD8 ratio for data analysis. The results are shown in Fig. 2. The absolute numbers of CD3-positive T lymphocytes and CD4-positive T lymphocytes were similar between the two groups, and there was no significant difference (Figs. 2A, 2B, p=0.1461 and p=0.9589, respectively), nonetheless, the patients with cervical cancer had considerably fewer $CD8^+$ T lymphocytes overall in their peripheral blood compared with the healthy controls (Fig. 2C, p=0.0057). When analyzing the percentage of the three T lymphocyte subsets, we found that the percentage of CD4-positive T lymphocytes did not differ between the two groups (Fig. 2E, p=0.8470), while the percentages of CD3-positive and CD8-positive T lymphocytes in the peripheral blood of the healthy individuals were higher

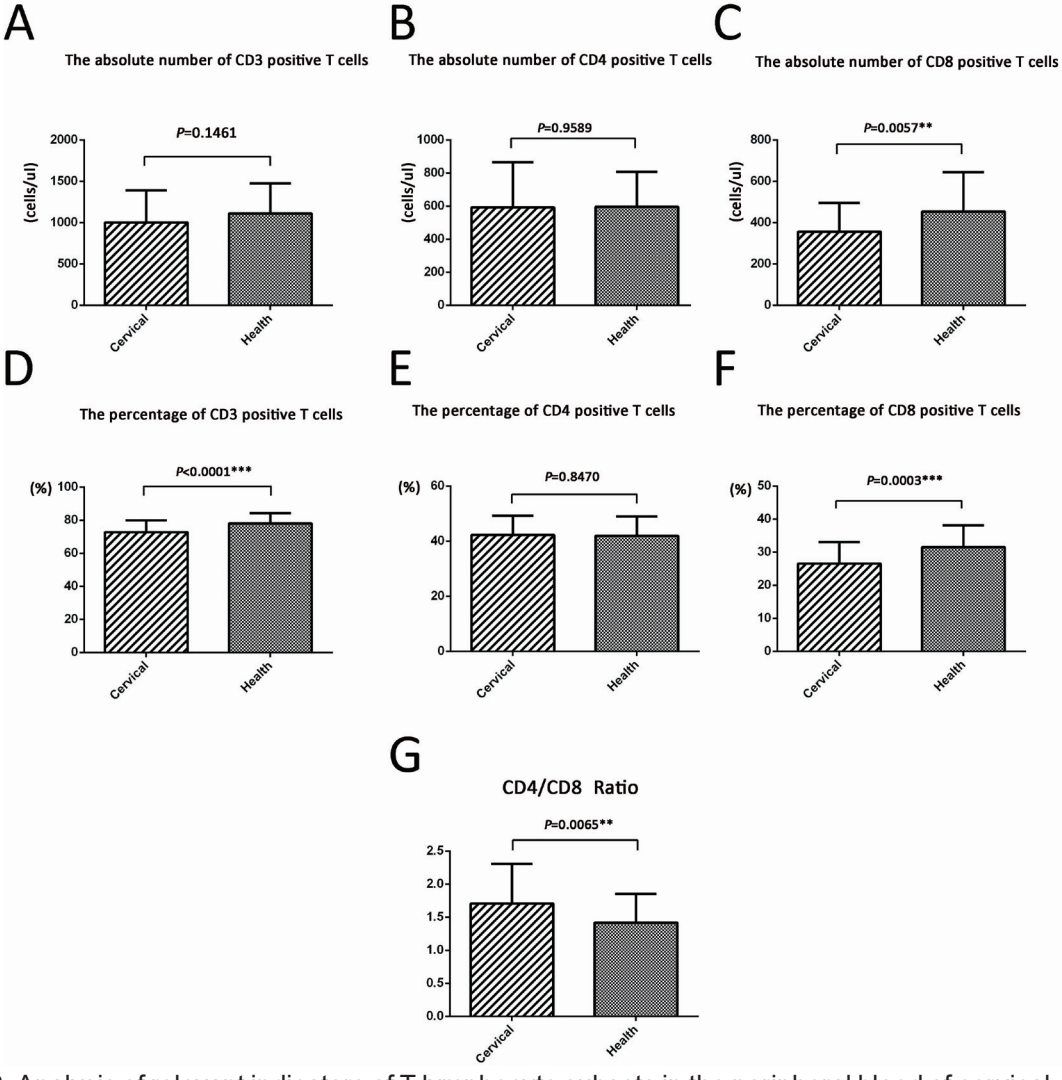

Fig. 2. Analysis of relevant indicators of T lymphocyte subsets in the peripheral blood of cervical cancer patients compared with the healthy people. A, B, C) Comparison of the absolute numbers of CD3-positive T cells, CD4-positive T cells and CD8-positive T cells in the peripheral blood of cervical cancer patients and the healthy people, respectively; D, E, F) Comparison of the percentage of CD3-positive T cells, CD4-positive T cells and CD8-positive T cells in the peripheral blood of cervical cancer patients and the healthy people, respectively; G) Comparison of the CD4/CD8 ratio in the peripheral blood of cervical cancer patients and the healthy people. The p-value shown, is obtained from the comparison between the indicated groups by nonparametric t-test. *p<0.05, **p<0.01, ***p<0.001

than that in the cervical cancer patients (Figs. 2D, 2F, $p<0.0001$ and $p=0.0003$, respectively). In addition, the percentage of $CD8^+$ T lymphocytes in the healthy individuals was higher than that in the patients with cervical cancer, which again confirms that healthy people have more CD8-positive T cells. The parameter CD4/CD8 ratio was observed in the healthy individuals, which was lower than that in the cervical cancer patients (Fig. 2G, $p=0.0065$). Therefore, we demonstrated that in the population of healthy people, the absolute number of CD8-positive T lymphocytes is significantly higher than that in the cervical cancer patients, and the CD4/CD8 ratio of healthy people is lower than that in cervical cancer patients.

Whether the absolute number of CD8 and the ratio of CD4/CD8 are related to tumor progression in patients with cervical cancer? To verify our hypothesis, this study combined and analyzed the seven indicators of the peripheral blood lymphocyte subsets of cervical cancer patients with their clinical information and conducted in-depth research and exploration.

The Relationship between the Peripheral Blood Lymphocyte Subsets and Clinical Information in Patients with Cervical Cancer

To further verify our hypothesis, we collected the clinical information of the 43 patients with cervical cancer, with an age range of 25-69 years, and a pathological type of squamous cell carcinoma. The clinical data of the patients and the seven T lymphocyte subset indicators in the peripheral blood were analyzed in this study.

Our study suggest that these five parameters (including the percentage of CD3-positive, CD4-positive and CD8-positive T lymphocytes and the absolute numbers of CD3-positive and CD4-positive T lymphocytes) were not related with FIGO stage, lymph node metastasis and distant metastasis in patients with cervical cancer. However, the absolute count of CD8-positive T lymphocytes and the CD4/CD8 ratio of lymphocyte subsets are related to the clinical FIGO stage, lymph node metastasis, and distant metastasis of cervical cancer patients, shown in Fig. 3 and Table 1. The total number of CD8-positive T lymphocytes in the peripheral blood of cervical cancer patients with lymph node metastasis and distant metastasis was less than that in patients without lymph node and distant metastasis

Table 1. Correlation analysis between the absolute number of CD8-positive T cells and the CD4/CD8 ratio in the peripheral blood of patients with cervical cancer and clinical characteristics

	Patients	Absolute number of CD8-positive T cells	*p*-value	CD4/CD8 Ratio	*p*-value
Age					
≤60	31	315.0(240.0, 455.0)	0.0899	1.700(1.300, 2.000)	0.8306
>60	12	420.0(308.5, 502.0)		2.150(1.400, 2.150)	
FIGO Stage					
I	8	335.0(299.0, 414.0)	0.2254	1.700(1.600, 1.800)	0.0414
II	15	286.0(215.0, 527.0)		1.400(0.950, 2,075)	
III	15	416.5(326.3, 510.3)		1.500(1.300, 2.000)	
IV	5	295.0(227.0, 348.5)		2.000(1.750, 3.100)	
Lymph node metastasis					
N0	22	413.5(295.8, 530.5)	0.0163	1.600(1.200, 1.800)	0.0411
N1	21	296.0(215.5, 411.0)		1.900(1.400, 2.100)	
Distant metastasis					
M0	39	376.0(285.5, 456.5)	0.0215	1.450(1.225, 1.800)	0.0311
M1	4	227.0(168.5, 292.3)		1.900(1.650, 2.600)	

FIGO: Federation International of Gynaecology and Obstetrics; N0: Cervical cancer patients without lymphatic invasion; N1: Cervical cancer patients with lymphatic invasion; M0: Cervical cancer patients without distant metastasis; M1: Cervical cancer patients with distant metastasis

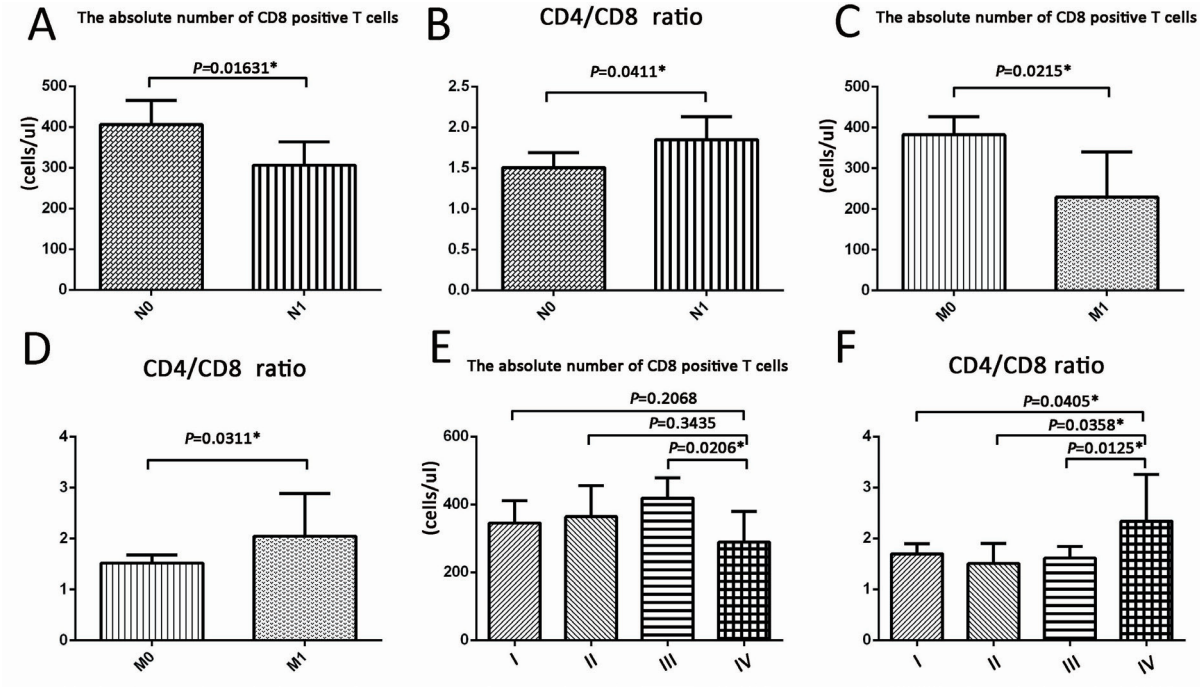

Fig. 3. The correlation between the absolute number of CD8-positive T cells and the CD4/CD8 ratio in the peripheral blood of cervical cancer patients, with FIGO stage, lymph node metastasis and distant metastasis. A) The correlation between the absolute number of CD8-positive T cells with lymph node metastasis; B) The correlation between the CD4/CD8 ratio with lymph node metastasis; C) The correlation between the absolute number of CD8-positive T cells with distant metastasis; D) The correlation between the CD4/CD8 ratio with distant metastasis; E) The correlation between the absolute number of CD8-positive T cells with FIGO stage; F) The correlation between the CD4/CD8 ratio with FIGO stage. FIGO, Federation International of Gynaecology and Obstetrics; N0, Cervical cancer patients without lymphatic invasion; N1, Cervical cancer patients with lymphatic invasion; M0, Cervical cancer patients without distant metastasis; M1, Cervical cancer patients with distant metastasis. The *p*-value shown is obtained from the comparison between the indicated groups by nonparametric t-test. **p*<0.05, ***p*<0.01, ****p*<0.001

(Figs. 3A, 3C, p=0.01631 and p=0.0215, respectively), and the CD4/CD8 ratio was significantly higher (Figs. 3B, 3D, p=0.0411 and p=0.0311, respectively). According to a univariate analysis, we found that the ratio of CD4/CD8 was related to the FIGO stage (p=0.0414), but the absolute number of CD8-positive T lymphocytes was not significantly different from the FIGO stage (p=0.2254, Table 1). Further analysis of the correlation between the absolute count of CD8-positive T lymphocytes and the CD4/CD8 ratio of patients with stage I-IV cervical cancer showed that the number of CD8-positive T lymphocytes of patients with stage IV cervical cancer was less than that in stage III patients (as shown in Fig. 3E, p=0.0206, there was a significant difference) and stage I-II cervical cancer (as shown in Fig. 3E, p=0.2068 and p=0.3435, respectively, there was no statistical significance, likely due to insufficient sample number). Stage IV patients had a considerably higher CD4/CD8 ratio than stage I-III individuals (Fig. 3F, p=0.0405, p=0.0358, and p=0.0123, respectively). Therefore, patients with early and mid-stage cervical cancer have more CD8-positive T cells and a lower CD4/CD8 ratio than in patients with advanced cervical cancer. Patients with lymph node and distant metastasis from cervical cancer have fewer CD8-positive T lymphocytes in their peripheral blood than in patients without this metastasis, and the CD4/CD8 ratio is higher in these individuals.

The Relationship between the CD39 Expression of T Cells in Peripheral Blood and Clinical Information in Cervical Cancer Patients

Adenosine is an important negative regulatory molecule in the tumor

microenvironment that exerts certain regulatory effects on immunity. What role does the CD39 molecule, as a critical molecule of the adenosine metabolic pathway, play in the immune system? This study detected CD39 expression on the T cell surface in the peripheral blood of cervical cancer patients and conducted a correlation analysis with clinical information.

The findings demonstrated that the expression of CD39 molecules in CD4 and CD8-positive T cells was related to clinical FIGO stage, lymph node metastasis, and distant metastasis in cervical cancer patients, as shown in Fig. 4. Patients with lymph node metastasis and distant metastasis had significant higher levels of CD39 expression in either CD4-positive or CD8-positive T cells than in those without lymph node metastasis and distant metastasis (Figs. 4A-4D, $p<0.0001$, $p<0.0001$, $p=0.0014$, and $p<0.0001$, respectively). In addition, CD39 expression was also associated with cervical cancer FIGO stage, with stage I showing the lowest expression level and stage IV showing the highest expression (Figs. 4E, 4F). Therefore, we suppose that patients with early and medium-term cervical cancer should have much lower CD39 expression in their T lymphocytes than in patients with more advanced cervical cancer.

Combined with the previous conclusions, we found that the number of CD8-positive T lymphocytes in patients with advanced cervical cancer was lowered, resulting in tumor progression and metastasis, in turn

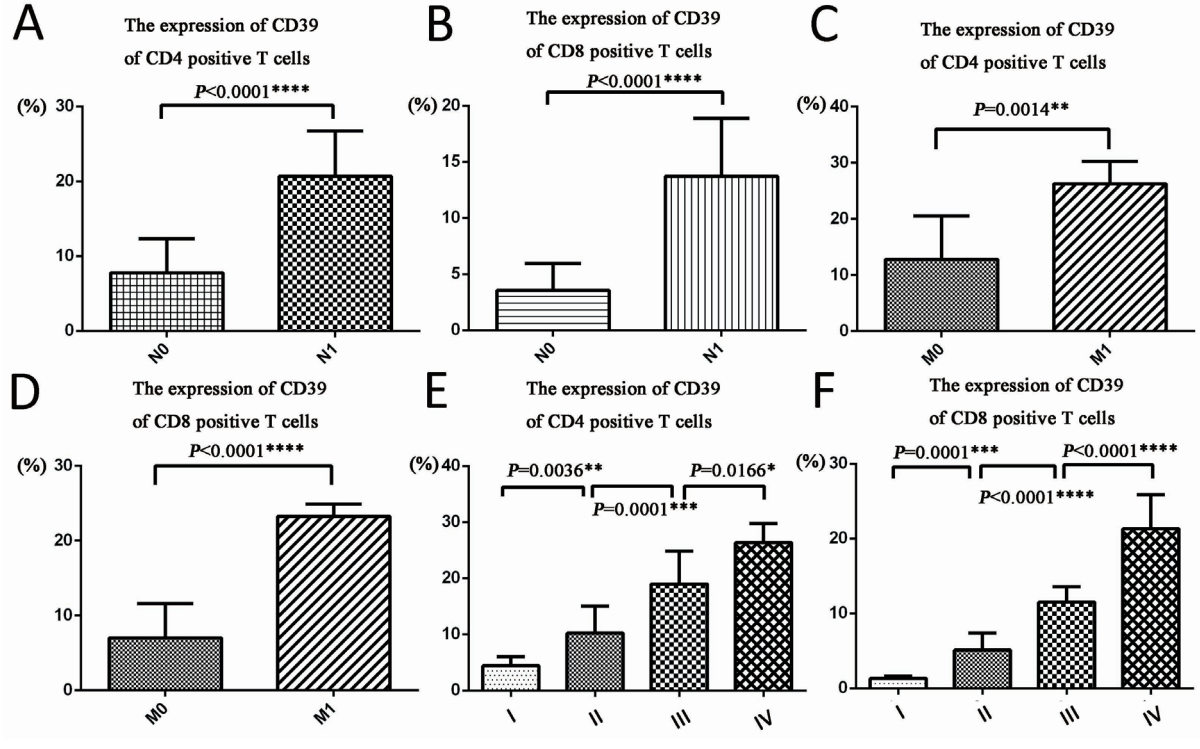

Fig. 4. Correlation between the expression of CD39 in T lymphocytes in the peripheral blood of cervical cancer patients with FIGO stage, lymph node metastasis and distant metastasis. A) The correlation between the expression of CD39 of CD4-positive T cells with lymph node metastasis; B) The correlation between the expression of CD39 of CD8-positive T cells with lymph node metastasis; C) The correlation between the expression of CD39 of CD4-positive T cells with distant metastasis; D) The correlation between the expression of CD39 of CD8-positive T cells with distant metastasis; E) The correlation between the expression of CD39 of CD4-positive T cells with FIGO stage; F) The correlation between the expression of CD39 of CD8-positive T cells with FIGO stage. FIGO, Federation International of Gynaecology and Obstetrics; N0, Cervical cancer patients without lymphatic invasion; N1, Cervical cancer patients with lymphatic invasion; M0, Cervical cancer patients without distant metastasis; M1, Cervical cancer patients with distant metastasis. The *p*-value shown is obtained from the comparison between the indicated groups by nonparametric t-test. *$p<0.05$, **$p<0.01$, ***$p<0.001$

causing poor prognosis. The percentage of $CD39^+$ T cells in patients with advanced cervical cancer was significantly higher than in those with early or medium-term cervical cancer. Our study demonstrated that the percentage of $CD8^+CD39^+$ T cells exhibits a negative correlation with the number of CD8-positive T lymphocytes (Fig. 5, r=-0.3777, p=0.0125). In conjunction with the existing research, this study analyzed the correlation between the absolute number of T lymphocytes and the expression of CD39 on T lymphocytes with clinical information for cervical cancer patients. This study proposed and verified the correlation between CD39 expression on T cells and clinical FIGO stage, lymph node metastasis, and distant metastasis in cervical cancer patients.

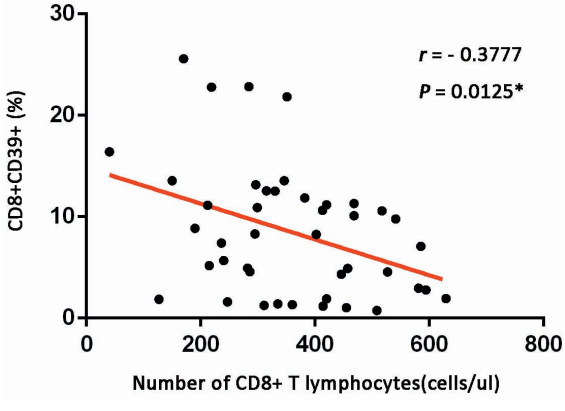

Fig. 5. The negative correlation between the percentage of $CD8^+CD39^+$ T cells with the number of CD8-positive T lymphocytes. The p-value shown is obtained from the analysis between the indicated groups by Person correlation analysis. *p<0.05, **p<0.01, ***p<0.001

DISCUSSION

The immune system is an important system that generates the immune response and manifests immune function, with the roles of monitoring, defense, and regulation (14). The immune system not only recognizes the pathogens of foreign invasion, but also identifies and removes other hazardous components, such as tumor cells and aging cells *in vivo*. Under normal circumstances, mutated tumor cells are identified and removed by the body`s immune system, but tumor cells in cancer patients escape the immune monitoring of the body due to the complexity of the tumor microenvironment, thereby promoting tumor advancement and even metastasis. How to effectively identify tumor cells and remove them has become one of the core challenges of cancer treatment (15, 16).

The immune system consists of a variety of components, including immune organs, immunocytes, and immune molecules. Lymphocytes serve as the main immunocytes and play important roles in the immune system (5). Lymphocytes can be divided into CD3-positive T cells, NK cells, and B cells, wherein CD3-positive T cells can be divided into CD4 and CD8-positive T cells. The CD4-positive T cells can be further divided into helper T, memory T, and regulatory T subsets (17, 18). Furthermore, macrophages, dendritic cells (DCs), and myeloid-derived suppressor cells (MDSCs) are also important immunocytes that assist in the immune system. The tumor microenvironment is a very complex internal environment, including not only cells such as tumor cells, immunocytes, and endothelial cells but also various cytokines and matrix components. Due to the complexity of immunocytes in the tumor environment, we cannot detect all immunocytes in real-time, so we used flow cytometry to detect CD3, CD4, CD8-positive T cells, NK cells, and B cells. The immune function of patients is evaluated by flow cytometry, thereby providing useful information for the clinic (19, 20).

T lymphocyte-mediated cellular immunity is an active immune response in the body. T lymphocytes perform a number of biological tasks, including killing target cells directly, assisting B cells in the production of antibodies, and generating cytokines (21-23). CD8-positive T cells are cytotoxic T cells that directly kill target cells (24). This study detected and analyzed T lymphocytes and their subsets. The results show that the absolute count of CD8-positive T lymphocytes and the CD4/CD8 ratio associated with cytotoxic T

cells in patients` peripheral blood, are related to clinical information. Our study suggest that absolute number is a better parameter than the percentage, because there is no relationship between the percentages of CD8-positive T lymphocytes with patients` FIGO stage, lymph node metastasis and distant metastasis. The total numbers of cytotoxic T cells in peripheral blood in tumor patients with early and medium-stage are higher than in those with the advanced stage suggesting that the number of cytotoxic T cells is related to prognosis, and patients who have more cytotoxic T cells in peripheral blood have a better prognosis. This work has effectively explained that cytotoxic T lymphocytes are the main performers of the body`s immune system to kill tumor cells only when the lymphocyte base reaches a certain extent. If the number of CD8-positive T lymphocytes is very low, the immune system cannot kill tumor cells, also affecting subsequent treatment, including some immunogenic anti-treatments, such as PD-1 and cytotoxic T-lymphocyte antigen 4 (CTLA-4) (25). Although peripheral blood lymphocyte subsets cannot accurately react to lymphocyte subsets in the tumor and tumor microenvironment, peripheral blood samples are convenient and accurate. In addition, the lymphocytes in the periphery and tumor are consistent, making peripheral blood lymphocyte subsets the most feasible assessment of the immune state of tumor patients (26-28).

We discovered a correlation between the absolute number of CD8-positive T lymphocytes in tumor patients and FIGO stage, lymph node metastasis, and distant metastasis, suggesting that this index is one of the independent factors of prognosis. We found that the absolute number of CD8-positive T cells in cervical cancer patients is correlated with patient tumor progression, and there are fewer CD8-positive T lymphocytes in advanced patients. So, the immune function of patients is weak, being more conductive to the progression and metastasis of tumors, and these patients also have a worse prognosis. The ratio of CD4/CD8, associated with the absolute count of CD8-positive T lymphocytes, is capable of reflecting the immunological function of cervical cancer patients, prompting prognosis. The CD4/CD8 ratio and absolute count of CD8-positive T lymphocytes together provide clinical information to promote follow-up treatment of patients. These two indicators are related to the tumor progression and metastasis, also independent factors for the prognosis of tumor patients. Patients with more CD8-positive T cells (relatively low CD4/CD8 ratios) in peripheral blood have a better prognosis. The more CD8-positive T lymphocytes there are in the peripheral blood, the better prognosis of patients. Additionally, our research revealed a correlation between clinical FIGO stage, lymph node metastasis, distant metastasis, and CD39 expression on T cells in cervical cancer patients. Patients with advanced cervical cancer should have significantly higher levels of CD39 expression in their T cells compared with those with early and medium-stage cervical cancer.

CD39 is the key molecule in adenosine metabolic pathways and is a negative immune regulatory factor. As a result, CD39 may also have the characteristics of negative immune regulation, which can cause the exhaustion of CD8-positive T cells by certain mechanisms or lead to a decrease in the number of CD8-positive T lymphocytes, ultimately leading to tumor progression and metastasis. One study reported that the CD39 expression can identify whether CD8-positive T lymphocytes are exhausted, and other studies have also confirmed that CD39 and PD-1 can be co-expressed at the T lymphocyte surface. CD39 molecules can also induce regulatory T cell transformation and formation (1). Combined with the discoveries of this study, we can suppose that CD39 expressed on the surface of CD8-positive T lymphocytes, as a negative regulatory molecule, can cause the exhaustion of CD8-positive T cells by certain signaling pathways, resulting in a corresponding reduction in the number of

CD8-positive T lymphocytes. In addition, CD39 can also further initiate the expression of some immune checkpoints in T cells, such as PD-1, therefore exerting a negative immune regulation function.

We hope that by detecting the lymphocyte subsets of the peripheral blood in tumor patients, it would be possible to provide more valuable information for clinics, and that a clinical treatment strategy can be developed according to the immune function state of each patient. Finally, patients can obtain optimized benefits from tests. In addition, this study also found that the CD39 expression and CD8-positive T lymphocytes showed a negative correlation, providing a basis for subsequent clarification of CD39 as a negative regulator.

ACKNOWLEDGMENTS

Funding: This work was supported by grants from Tianjin Medical University Cancer Institute & Hospital College-level Research Seed Fund (No. 1907), the National Natural Science Foundation of China (No. 81602020).

AUTHORS' CONTRIBUTION

Concept - Hao Jin; Design - Hao Jin; Supervision - Hao Jin; Resources - Kuo Zhao, Dongmei Han, Lu Tang; Materials - Kuo Zhao, Dongmei Han, Lu tang; Data Collection and Processing - Kuo Zhan, Hao Jin; Analysis and Interpretation - Kuo Zhao, Hao Jin; Literature Search - Kuo Zhao; Writing Manuscript - Hao Jin; Critical Review - Kuo Zhao, Hao Jin;

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Yang L, Zheng R, Wang N, Yuan Y, Liu S, Li H, et al. Incidence and mortality of stomach cancer in China, 2014. Chin J Cancer Res. 2018;30(3):291-8.
2. Diefenbach D, Greten HJ, Efferth T. Genomic landscape analyses in cervical carcinoma and consequences for treatment. Curr Opin Pharmacol. 2020;54:142-57.
3. Fahmi T, Esendagli G, Yilmaz G, Kansu E, Guc D. Immune compartmentalization of T cell subsets in chemically-induced breast cancer. Scand J Immunol. 2010;72(4):339-48.
4. Yoshimura K, Laird LS, Chia CY, Meckel KF, Slansky JE, Thompson JM, et al. Live attenuated Listeria monocytogenes effectively treats hepatic colorectal cancer metastases and is strongly enhanced by depletion of regulatory T cells. Cancer Res. 2007;67(20):10058-66.
5. Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol. 2016;17(8):e328-e46.
6. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991-8.
7. Xia Y, Li W, Li Y, Liu Y, Ye S, Liu A, et al. The clinical value of the changes of peripheral lymphocyte subsets absolute counts in patients with non-small cell lung cancer. Transl Oncol. 2020;13(12):100849.
8. Showe MK, Kossenkov AV, Showe LC. The peripheral immune response and lung cancer prognosis. Oncoimmunology. 2012;1(8):1414-6.
9. Allard B, Longhi MS, Robson SC, Stagg J. The ectonucleotidases CD39 and CD73: Novel checkpoint inhibitor targets. Immunol Rev. 2017;276(1):121-44.
10. Magid-Bernstein JR, Rohowsky-Kochan CM. Human CD39(+) Treg Cells Express Th17-Associated Surface Markers and Suppress IL-17 via a Stat3-Dependent Mechanism. J Interferon Cytokine Res. 2017;37(4):153-64.
11. Bai A, Robson S. Beyond ecto-nucleotidase: CD39 defines human Th17 cells with CD161. Purinergic Signal. 2015;11(3):317-9.
12. Figueiro F, Muller L, Funk S, Jackson EK, Battastini AM, Whiteside TL. Phenotypic and functional characteristics of CD39(high) human regulatory B cells (Breg). Oncoimmunology. 2016;5(2):e1082703.
13. Tondell A, Wahl SGF, Sponaas AM, Sorhaug S, Borset M, Haug M. Ectonucleotidase CD39 and Checkpoint Signalling Receptor Programmed Death 1 are Highly Elevated in Intratumoral Immune Cells in Non-small-cell Lung Cancer. Transl Oncol. 2020;13(1):17-24.

14. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140(6):883-99.
15. Yang F, Jin H, Wang J, Sun Q, Yan C, Wei F, et al. Adoptive Cellular Therapy (ACT) for Cancer Treatment. Adv Exp Med Biol. 2016;909:169-239.
16. Jin H, Sun L, Tang L, Yu W, Li H. Expression of GARP Is Increased in Tumor-Infiltrating Regulatory T Cells and Is Correlated to Clinicopathology of Lung Cancer Patients. Front Immunol. 2017;8:138.
17. Wang YY, Zhou N, Liu HS, Gong XL, Zhu R, Li XY, et al. Circulating activated lymphocyte subsets as potential blood biomarkers of cancer progression. Cancer Med. 2020;9(14):5086-94.
18. Li N, Zhang L, Song HL, Zhang J, Weng HW, Zou LQ. Prognostic impact of absolute lymphocyte count/absolute monocyte count ratio and prognostic score in patients with nasal-type, extranodal natural killer/T-cell lymphoma. Tumour Biol. 2017;39(5):1010428317705503.
19. Kuss I, Hathaway B, Ferris RL, Gooding W, Whiteside TL. Decreased absolute counts of T lymphocyte subsets and their relation to disease in squamous cell carcinoma of the head and neck. Clin Cancer Res. 2004;10(11):3755-62.
20. Milne K, Alexander C, Webb JR, Sun W, Dillon K, Kalloger SE, et al. Absolute lymphocyte count is associated with survival in ovarian cancer independent of tumor-infiltrating lymphocytes. J Transl Med. 2012;10:33.
21. Ahrends T, Spanjaard A, Pilzecker B, Babala N, Bovens A, Xiao Y, et al. CD4(+) T Cell Help Confers a Cytotoxic T Cell Effector Program Including Coinhibitory Receptor Downregulation and Increased Tissue Invasiveness. Immunity. 2017;47(5):848-61 e5.
22. Borst J, Ahrends T, Babala N, Melief CJM, Kastenmuller W. CD4(+) T cell help in cancer immunology and immunotherapy. Nat Rev Immunol. 2018;18(10):635-47.
23. Melssen M, Slingluff CL, Jr. Vaccines targeting helper T cells for cancer immunotherapy. Curr Opin Immunol. 2017;47:85-92.
24. Chang WC, Li CH, Huang SC, Chang DY, Chou LY, Sheu BC. Clinical significance of regulatory T cells and CD8+ effector populations in patients with human endometrial carcinoma. Cancer. 2010;116(24):5777-88.
25. Dovsak T, Ihan A, Didanovic V, Kansky A, Verdenik M, Hren NI. Effect of surgery and radiotherapy on complete blood count, lymphocyte subsets and inflammatory response in patients with advanced oral cancer. BMC Cancer. 2018;18(1):235.
26. Riemann D, Cwikowski M, Turzer S, Giese T, Grallert M, Schutte W, et al. Blood immune cell biomarkers in lung cancer. Clin Exp Immunol. 2019;195(2):179-89.
27. Xu YF, Lu Y, Cheng H, Shi S, Xu J, Long J, et al. Abnormal distribution of peripheral lymphocyte subsets induced by PDAC modulates overall survival. Pancreatology. 2014;14(4):295-301.
28. Oh SY, Heo J, Noh OK, Chun M, Cho O, Oh YT. Absolute Lymphocyte Count in Preoperative Chemoradiotherapy for Rectal Cancer: Changes Over Time and Prognostic Significance. Technol Cancer Res Treat. 2018;17:1533033818780065.