Expression and Clinical Significance of Peripheral Blood Tim3 and Programmed Cell Death Protein 1 in Patients with Colon Cancer

AIHUA WANG AND YONGXIANG MA*

Department of Oncology, Anqiu Hospital of Traditional Chinese Medicine, Anqiu, Shandong 262100, China

To found the clinical role of T cell immunoglobulin and mucin domain-containing protein 3 and programmed cell death protein 1 expression from the peripheral blood in patients with colon cancer is the objective of the study. Total of 98 colon cancer patients from our hospital between January 2020 and March 2022 were considered as the study group. An additional 86 healthy subjects from our hospital were considered as the healthy group. Spearman correlation analysis was used to detect the clinical features of the patients and receiver operating characteristic curve were used to detect the diagnosis values of the indexes. The blood expression of T cell immunoglobulin and mucin domain-containing protein 3^+, programmed cell death protein 1^+ and T cell immunoglobulin and mucin domain-containing protein 3^+ programmed cell death protein 1^+ were significantly higher in the study group than that in healthy group ($p<0.05$). Although, there was no significant difference in the expression of T cell immunoglobulin and mucin domain-containing protein 3^+, programmed cell death protein 1^+ and T cell immunoglobulin and mucin domain-containing protein 3^+ programmed cell death protein 1^+ in relation to tumor location or distant metastasis ($p>0.05$), but there was a positive correlation with tumor size and tumor, nodes and metastases stage. The combined diagnosis of peripheral blood T cell immunoglobulin and mucin domain-containing protein 3^+, programmed cell death protein 1^+ and T cell immunoglobulin and mucin domain-containing protein 3^+ programmed cell death protein 1^+ showed higher area under the curve=0.964 than a single index examination ($p<0.05$). Higher T cell immunoglobulin and mucin domain-containing protein 3^+, programmed cell death protein 1^+ and T cell immunoglobulin and mucin domain-containing protein 3^+ programmed cell death protein 1^+ cells in peripheral blood can provide a basis for clinical diagnosis and progression of colon cancer.

Key words: T cell immunoglobulin and mucin domain-containing protein 3, programmed cell death protein 1, colon cancer, Spearman correlation analysis

Colon cancer is a highly prevalent tumor in the digestive system. The onset of cancer involve intricate biological changes that occur at various stages and involve multiple factors, with a significant correlation to the immune system function of the body[1,2]. It has been reported that the low or absence of immune function of tumor cells is a key factor in accelerating malignant progression[3]. T cell immunoglobulin and mucin domain-containing protein 3 (Tim3) and Programmed Cell Death protein 1 (PD1) are common surface molecules on T cells, which negatively regulated the immune function[4,5]. Blocking Tim3 and PD1 can promote the gradual recovery of T lymphocyte function and their ability to prevent tumor invasion. However, there is a paucity of research on the changes in Tim3 and PD1 expression in the peripheral blood of colon cancer patients to detect the relationship of them with clinical features in colon cancer patients. 98 colon cancer patients in the department of tumor proctology from January 2020 to March 2022 were collected as the study group. The inclusion criteria include patients age≥50 y; confirmed diagnosis of primary colon cancer by pathological examination; expected survival time>3 mo. The exclusion criteria include other malignant tumors; other systemic severe diseases and incomplete clinical data. Total of 98 patients, with 63 males and 35 females, aged 50 to 77 y and average age (65.67±5.74) y were set as the study group. Among them 37 patients had lymph node metastasis and the remaining 61 patients doesn't have lymph node metastasis. The histological

***Address for correspondence**
E-mail: 763681265@qq.com

classification includes 69 patients of tubular adenocarcinoma and 29 patients of mucinous adenocarcinoma. The Tumor, Nodes and Metastases (TNM) stage was I-II in 52 patients and III-IV in 46 patients. Tumor diameter was <5 cm in 73 patients and >25 cm in 25 patients. In addition, at the same time, we also collected the 86 healthy individuals as the healthy group who underwent physical examination, aged 51 to 76 y, with an average age of 65.75±5.64 y, among which 60 males and 26 females. All patients participating in this study volunteered and signed informed consent. This study was approved by the Ethics Committee of Anqiu Hospital of Traditional Chinese Medicine. Test methods of $PD1^+$ and Tim3 used in the study was explained here. On the 2nd d following admission, in the morning peripheral blood samples (2 ml) under fasting condition were collected in sterile centrifuge tubes. These blood samples were then diluted with sterile Phosphate Buffered Saline (PBS) at a 1:1 ratio, followed by repeated pipetting and centrifugation. Next, lymphocyte separation solution was added to the centrifuge tubes at a 2:1 ratio and the samples were subjected to centrifugation at 1800 rpm for 25 min. The intermediate white lymphocyte membrane layer was obtained and transferred to a preloaded 15 ml PBS centrifuge tube, which was then centrifuged at 1800 rpm for 5 min. After discarding the PBS, the cells were resuspended in 15 ml of PBS and Peripheral Blood Mononuclear Cells (PBMCs) were obtained *via* centrifugation at 1200 rpm for 5 min. $Tim3^+$ and $PD1^+$ cells were detected using Flow Cytometry Standard (FCS). To detect $Tim3^+PD1^+$ cells, the fluorescence compensation value between Tim3-Phycoerythrin (PE) and PD1-Allophycocyanine (APC) was adjusted by the instrument and $CD3^+$ cells were gated. The gated double-positive $Tim3^+PD1^+$ cells in $Tim3^+$ were then calculated. Statistical Package for the Social Sciences (SPSS) 21.0 and FlowJo™ software were used for the study statistical analysis. Category variables data were expressed as the number (%) and the chi-square test was used for inter-group comparison. The continuous data were displayed as mean±standard deviation and the Least Significant Difference (LSD) test and t-test were used to calculate their differences. The Spearman correlation was used for correlation analysis and $p<0.05$ was set as statistically significant. Comparison of $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ expression differences between two groups were shown in Table 1. After FCS detection, we found that $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ cells percentage of the study group were all higher than those in healthy group ($p<0.05$). Relationship of $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ with different clinical features in colon cancer patients were shown in Table 2. The $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ cell percentage had no significant difference in expression among the groups of patient's tumor location and presence of distant metastasis (all $p>0.05$). However, the number of $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$cells was statistically significant with respect to TNM stage and tumor size ($p<0.05$) in colon cancer patients. We then conducted the Spearman analysis which showed that tumor size and TNM stage were positively correlated with the number of $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ cells ($p<0.05$). The higher the tumor size and TNM stage, the higher the number of $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ cells as shown in Table 3. Diagnostic values of peripheral blood cells in colon cancer were shown in Table 4. The Area Under the Curve (AUC=0.964) of combined diagnosis of peripheral blood $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ cells were higher than that of single indicator examination ($p<0.05$). It is reported in the literature that the total effective rate of colon cancer treatment is less than 70 % and the clinical remission rate of surgery combined with radiotherapy and chemotherapy is not high[6,7]. Previous literature has also pointed out that there is no screening index with diagnostic value in the early stage of colon cancer. Although epithelial tumor glycoprotein components such as carcinoembryonic antigen and Glycoprotein-125 (CA-125) can enhance the diagnostic rate of colon cancer, that lack specificity and sensitivity, and are insufficient as diagnostic indicators for colon cancer in clinical practice[8-10]. The pathogenesis of colon cancer is complex and the decline of immune function or immune blockade is one of the key factors to induce tumors and promote disease progression[11,12]. T lymphocytes secrete a large number of cytokines after being activated by stimulation signals and release granzyme and perforin, which act on tumors through different death receptor pathways. Scholars have found that costimulatory molecule B and T Lymphocyte Attenuator (BTLA) and its ligand herpes virus invasion mediator participate in negative regulation of T cell differentiation and proliferation expression, and promote the decline of immune function of tumor cells[13,14]. As negative co-stimulatory molecules in T lymphocytes, Tim3 and PD1 are detected in different immune cell surfaces, such as T cells, monocytes,

macrophages, dendritic cells and mast cells. They play a role when the body is invaded by inflammatory factors or undergoes stress reactions. Moreover, Tim3 and PD1 can be regulated in different tumor microenvironments. When Tim3 and PD1 bind to their corresponding ligands, T cells undergo decline, regulatory T cells increase immunosuppression and tumor-associated macrophages tend to polarize to the M2 subtype, creating a microenvironment for tumor survival and causing immunosuppression of tumor cells[15,16]. Previous studies showed that $Tim3^+$ and $PD1^+$ are elevated in the peripheral blood of various cancers, such as colon cancer, gastric cancer and cervical cancer, and are closely related to tumor development and patient prognosis[17-19]. This study compared the differences of the number of blood Tim3 and PD1 cells between colon cancer patients and healthy people. Detected results showed that $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ were significantly higher in study group than in healthy group[20,21]. This study also analyzed the correlation between different pathological characteristics and $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ cells number. The findings revealed that tumor size and TNM stage were positively correlated with $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$. The higher the TNM stage and the larger the tumor size, the higher the expression of $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ cells. Therefore, in patients with colon cancer at an intermediate stage, high expression of $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ may act on T lymphocytes to inhibit their immune surveillance and immune clearance capabilities, ultimately leading to colon cancer occurrence and development[22-24].

TABLE 1: $Tim3^+$, $PD1^+$ AND $Tim3^+PD1^+$ EXPRESSION IN THE TWO GROUPS

Groups	n	$Tim3^+$ (%)	$PD1^+$ (%)	$Tim3^+PD1^+$
Study group	98	87.41±2.85	82.34±7.65	57.89±6.54
Healthy group	86	34.35±2.72	21.57±6.57	18.62±5.39
t	-	128.709	57.395	44.074
p	-	<0.001	<0.001	<0.001

TABLE 2: RELATIONSHIP OF $Tim3^+$, $PD1^+$ AND $Tim3^+PD1^+$ CELLS PERCENTAGES WITH DIFFERENT CLINICAL FEATURES IN COLON CANCER PATIENTS

Variables	n	$Tim3^+$ (%)	$PD1^+$ (%)	$Tim3^+PD1^+$
Location				
Rectal	48	68.34±6.25	75.14±9.55	42.64±5.25
Colon	50	67.01±5.84	74.73±8.97	40.86±4.74
t		1.089	0.219	1.763
p		0.279	0.827	0.081
Metastasis				
Yes	37	71.34±9.25	74.81±10.12	44.51±5.83
No	61	68.63±8.21	72.11±9.74	43.61±4.67
t		1.51	1.311	0.841
p		0.134	0.193	0.402
TNM stage				
I-II	52	62.74±10.64	62.35±8.65	57.81±5.66
III-IV	46	83.31±9.52	81.63±10.47	41.43±7.25
t		10.032	9.978	12.538
p		<0.001	<0.001	<0.001
Tumor size, cm				
<5	73	69.24±8.97	62.62±10.52	37.78±4.18
≥5	25	84.67±9.64	81.95±8.41	57.53±5.64
t		7.026	8.313	16.042
p		<0.001	<0.001	<0.001

TABLE 3: CORRELATION BETWEEN CLINICAL FEATURES AND THE NUMBER OF $Tim3^+$, $PD1^+$ AND $Tim3^+PD1^+$ CELLS

Variables	Tumor size		Tumor stage	
	r	p	r	p
$Tim3^+$	0.422	0.03	0.62	<0.001
$PD1^+$	0.644	0.025	0.942	<0.001
$Tim3^+PD1^+$	0.554	<0.001	0.928	<0.01

Note: r indicates correction coefficient and p indicates probability

TABLE 4: DETECTION OF DIAGNOSTIC VALUE FOR THE NUMBER OF PERIPHERAL BLOOD $Tim3^+$, $PD1^+$ AND $Tim3^+PD1^+$ CELLS IN COLON CANCER

Variables	Cut-off	Sensitivity	Specificity	AUC	95 % Confidence Interval (CI)
$Tim3^+$	64.83	75.5	74.4	0.754	0.683~0.842
$PD1^+$	79.28	64.2	76.7	0.697	0.621~0.756
$Tim3^+PD1^+$	43.22	66.3	73.2	0.704	0.652~0.764
Combination	-	91.8	93	0.964	0.920~0.988

In addition, this study found that the combined diagnosis of $Tim3^+$, $PD1^+$ and $Tim3^+PD1^+$ in peripheral blood had a higher AUC=0.964 than a single indicator alone. However, due to the limited number of patients, further research and analysis are needed. In summary, higher levels of Tim3 and PD1 in patient's blood with colon cancer may serve as an evaluation indicator for the occurrence and progression of colon cancer, especially in patients with larger tumor size and higher TNM stages.

Conflict of interests:

The authors declared no conflict of interest.

REFERENCES

1. Brauneck F, Weimer P, Schulze zur Wiesch J, Weisel K, Leypoldt L, Vohwinkel G, *et al.* Bone marrow-resident Vδ1 T cells co-express TIGIT with PD1, TIM-3 or CD39 in AML and myeloma. Front Med 2021;4(1):18-9.
2. Kato R, Jinnouchi N, Tuyukubo T, Ikarashi D, Matsuura T, Maekawa S, *et al.* TIM3 expression on tumor cells predicts response to anti-PD1 therapy for renal cancer. Transl Oncol 2021;14(1):918-22.
3. Dou K, Fan C, Feng W, Kong Y, Xiang Y, Wang Z, *et al.* "Dual-Lock-Dual-Key" controlled second near-infrared molecular probe for specific discrimination of orthotopic colon cancer and imaging-guided tumor excision. J Chin Chem Soc 2022;4(11):3609-26.
4. Lin H, Zhen H, Shan K, Ma X, Cao B. Alteration in the immune microenvironment based on APC status in MSS/pMMR colon cancer data retrieved from TCGA. Front Genet 2021;6(1):8-14.
5. Zhang ZN, Zhu ML, Chen YH, Fu YJ, Zhang TW, Jiang YJ, *et al.* Elevation of Tim-3 and PD1 expression on T cells appears early in HIV infection, and differential Tim-3 and PD1 expression patterns can be induced by common γ-chain cytokines. Biomed Res Int 2015;8(5):916-36.
6. Zheng X, Wang Q, Zhou Y, Zhang D, Geng Y, Hu W, *et al.* N-acetyltransferase 10 promotes colon cancer progression by inhibiting ferroptosis through N4-acetylation and stabilization of Ferroptosis Suppressor Protein 1 (FSP1) mRNA. Cancer Commun 2022;42(12):1347-66.
7. Chajuwan T, Kansuwan P, Kobbuaklee S, Chanswangphuwana C. Characteristics and clinical correlation of TIM-3 and PD1/PD-L1 expressions in leukemic cells and tumor microenvironment in newly diagnosed acute myeloid leukemia. Leuk Lymphoma 2021;63(2):450-6.
8. Klapholz M, Drage MG, Srivastava A, Anderson AC. Presence of $Tim3^+$ and PD-1^+ $CD8^+$ T cells identifies microsatellite stable colorectal carcinomas with immune exhaustion and distinct clinicopathological features. J Pathol 2022;257(2):186-197.
9. Kursunel MA, Taskiran EZ, Tavukcuoglu E, Yanik H, Demirag F, Karaosmanoglu B, *et al.* Small cell lung cancer stem cells display mesenchymal properties and exploit immune checkpoint pathways in activated cytotoxic T lymphocytes. Cancer Immunol Immunother 2021;7(1):1-15.
10. Roberts A, Bentley L, Tang T, Stewart F, Pallini C, Juvvanapudi J, *et al. Ex vivo* modelling of PD1/PD-L1 immune checkpoint blockade under acute, chronic and exhaustion-like conditions of T-cell stimulation. Sci Rep 2021;11(1):1-2.
11. Razmi M, Ghods R, Vafaei S, Sahlolbei M, Saeednejad Zanjani L, *et al.* Clinical and prognostic significances of cancer stem cell markers in gastric cancer patients: A systematic review and meta-analysis. Cancer Cell Int 2021;21(1):139.
12. Schulte S, Heide J, Ackermann C, Peine S, Ramharter M, Mackroth MS, *et al.* Deciphering the *Plasmodium falciparum* malaria-specific $CD4^+$ T-cell response: *Ex vivo* detection of high frequencies of $PD1^+TIGIT^+$ EXP1-specific $CD4^+$ T cells using a novel HLA-DR11-restricted MHC class II tetramer. Clin Exp Immunol 2021;207(2):227-36.
13. Yuan L, Huang T, Xu BL, Wang ZL, Run ZC, Zhao P, *et al.* Clinical significance of the programmed death-1^+ T cell immunoglobulin domain and mucin domain-3^+ frequency on $CD4^+$ T lymphocytes in patients with colorectal cancer. Chin J Exp Surg 2017;34(5):856-9.
14. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD1 pathways to reverse

T cell exhaustion and restore anti-tumor immunity. J Exp Med 2010;207(10):2187-94.

15. Sharifzad F, Yasavoli-Sharahi H, Mardpour S, Fakharian E, Nikuinejad H, Heydari Y, *et al.* Neuropathological and genomic characterization of glioblastoma-induced rat model: How similar is it to humans for targeted therapy? J Cell Physiol 2019;234(12):22493-504.
16. Li J, Ferris RL. Differential expression of PD1 and Tim-3 marks activation versus exhaustion status of T cells in the tumor microenvironment. J Immunother Cancer 2014;2(3):220.
17. Tian T, Li Z. Targeting Tim-3 in cancer with resistance to PD1/PD-L1 blockade. Front Oncol 2021;11(5):38-42.
18. Gallazzi M, Baci D, Mortara L, Bosi A, Buono G, Naselli A, *et al.* Prostate cancer peripheral blood NK cells show enhanced CD9, CD49a, CXCR4, CXCL8, MMP-9 production and secrete monocyte-recruiting and polarizing factors. Front Immunol 2021;11(2):58-66.
19. Bu X, Juneja VR, Reynolds CG, Mahoney KM, Bu MT, McGuire KA, *et al.* Monitoring PD1 phosphorylation to evaluate PD1 signaling during antitumor immune responses. Cancer Immunol Res 2021;9(12):1465-75.
20. Zhang X, Liu G, Shi X, Shi X, Li J, Mo L, *et al.* Sequential administration of anti-PD1 and anti-Tim-3 combined with an SA-GM-CSF-anchored vaccine overcomes adaptive immune resistance to reject established bladder cancer. J Cancer 2021;12(7):2000-9.
21. Muhammed MM, Elzawawy AM, Abozeid M, Soliman SH. Pretreatment neutrophil/lymphocytes ratio in non-metastatic colon cancer as a prognostic and predictive factor: A retrospective study. J Cancer Ther 2023;14(1):6-24.
22. Zanjani LS, Vafaei S, Abolhasani M, Fattahi F, Madjd Z. Prognostic value of Talin-1 in renal cell carcinoma and its association with B7-H3. Cancer Biomark 2022;35(3):1-24.
23. Kandel S, Adhikary P, Li G, Cheng K. The TIM3/Gal9 signaling pathway: An emerging target for cancer immunotherapy. Cancer Lett 2021;510(1):67-78.
24. Wang H, Gao Y, Vafaei S, Yu Q, Zhang J, Wang L. A chemoresistance lncRNA signature for recurrence risk stratification of colon cancer patients with chemotherapy. Mol Ther Nucleic Acids 2022;27:427-38.

This article was originally published in a special issue, "Innovations in Biomedical Research and Drug Development" Indian J Pharm Sci 2023:85(3) Spl Issue "150-154"