MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2017; 23: 4619-4632
DOI: 10.12659/MSM.904154

Received: 2017.03.04
Accepted: 2017.04.04
Published: 2017.09.26

Tumor-Infiltrating CD4+ Lymphocytes Predict a Favorable Survival in Patients with Operable Esophageal Squamous Cell Carcinoma

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BC 1,2 Kaiyan Chen*
CD 3 Ziyu Zhu*
DE 4 Nan Zhang
BE 5 Guoping Cheng
E 1,4 Fanrong Zhang
F 1,4 Jiaoyue Jin
EF 1 Junzhou Wu
FG 1 Lisha Ying
A 4 Weimin Mao
A 1 Dan Su

1 Cancer Research Institute, Zhejiang Cancer Hospital and Key Laboratory Diagnosis and Treatment Technology on Thoracic Oncology of Zhejiang Province, Hangzhou, Zhejiang, P.R. China
2 Department of Oncology, The Second Clinical Medical College of Zhejiang Chinese Medical University, Hangzhou, Zhejiang, P.R. China
3 School of Stomatology, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, P.R. China
4 Department of Oncology, The First Clinical Medical College of Wenzhou Medical University, Wenzhou, Zhejiang, P.R. China
5 Department of Pathology, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, P.R. China

* These authors contributed equally to this work

Corresponding Authors: Dan Su, e-mail: sudan@zjcc.org.cn; Weimin Mao, e-mail: maowm1218@163.com

Source of support: This study was supported by grants from the National Nature Science Foundation of China (No. 81472203 and No. 81602615), the Major Science and Technology Project of Medical and Health of Zhejiang Province of China (No. WKJ-ZJ-1403), the Major Science and Technology Project of Zhejiang Province of China (No. 2014C03029), and the 1022 program of Zhejiang Cancer Hospital

Background: The immune status within the tumor microenvironment has not been well determined in esophageal squamous cell carcinoma (ESCC). The aim of this study was to investigate the distributions of tumor-infiltrating T lymphocytes (TILs), and analyze their associations with clinical characteristics and prognosis; as well as investigate the expression of programmed death-ligand 1 (PD-L1) which has been identified as a favorable indicator of prognosis in our previous study on ESCC.

Material/Methods: Five hundred and thirty-six patients who underwent radical surgery for ESCC between January 2008 and April 2012 in Department of Thoracic Surgery at Zhejiang Cancer Hospital were included in the study. Immunohistochemistry was used to investigate the infiltration of various TILs (CD3+, CD4+, CD8+ T lymphocytes) in ESCC tissues. Chi-square test and Cox proportional hazards regression were used to explore the correlations between TILs abundance and clinicopathological variables and survival.

Results: The infiltration of intraepithelial CD4+ (iCD4+) lymphocytes was markedly higher than it in the stromal region (44.2% for intraepithelial versus 28.9% for stromal, $p<0.001$). Moreover, increased iCD4+ lymphocytes were significantly associated with longer overall survival (OS, $p=0.001$) in univariate analysis and were identified as an independent predictor for improved OS in multivariate analysis (hazard ratio [HR]=0.67, 95% confidence interval [CI]: 0.51–0.88, $p=0.040$). Neither the infiltration of CD3+ nor CD8+ lymphocytes showed the prognostic value in ESCC ($p>0.05$). Unexpectedly, combined with our previous study results, the TILs infiltration in ESCC showed an inverse association with the expression of PD-L1 ($p=0.027$).

Conclusions: Our results suggested that iCD4+ lymphocytes infiltration could be a favorable indicator for prognosis in ESCC.

MeSH Keywords: Antigens, CD4 • Esophageal Neoplasms • Immunochemistry • Survival Analysis • T-Lymphocytes, Helper-Inducer

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/904154

2607 8 2 44

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Esophageal cancer is the sixth most common cause of cancer-related deaths worldwide [1]. Approximately, 70% of global esophageal cancer cases occur in China, where it is the fourth cause of cancer death [2]. Esophageal squamous cell carcinoma (ESCC) accounts for more than 90% of esophageal cancer cases in Chinese patients [3]. Despite recent improvements in therapy, the outcome of ESCC remains dismal [4,5].

Recent advances in cancer immunological therapeutics have revealed the importance of signaling between programmed death-1 (PD-1), mainly expressed on antigen-experienced T cells, and its ligand PD-L1, expressed on tumor cells and antigen presenting cells [6]. The combination of PD-1 and PD-L1 is the key immune checkpoint receptor to inhibit T-cell activation [6], inducing impaired immune response and worse prognosis in various cancers [7,8]. Clinical trials have demonstrated that patients with malignant melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma benefit from anti-PD-1 and anti-PD-L1 therapy [9–11]. However, recent literature reported that patients with high expression of PD-1 and PD-L1 had better prognosis in breast cancer, metastatic melanoma, colorectal cancer, pulmonary squamous cell carcinoma, and ovarian cancer [12–16]. Moreover, only a fraction of patients respond to PD-1 or PD-L1 blockage [17,18]. Therefore, the role of PD-L1 as a predictive biomarker for immune treatment response in various solid tumors remains controversial [8,19,20].

We previously identified PD-L1 expression of 41.4% (222/536) in our ESCC cohort [21], and found that PD-L1 expression differed significantly by tumor location, grade, lymph node metastases, and disease stage [21]. Moreover, patients with positive PD-L1 expression had reduced risk for disease relapse compared to those without PD-L1 expression [21]. Our finding of an inverse association between the expression of this favorable predictor PD-L1 and lymph node metastasis prompted us to speculate that PD-L1 might be induced by an active immune response including T-cell proliferation.

High numbers of tumor-infiltrating lymphocytes (TILs) have been repeatedly shown to provide a significant survival advantage in ESCC. Particularly the presence of T cells (CD3+) and various T cell subpopulations (e.g., CD4+, CD8+, CD103+) have been shown to be indicators of a better prognosis [22,23]. Considering that PD-L1 expression could be induced by activated T cells [24,25], we next explored the potential association between PD-L1 expression and TIL abundance in ESCC.

In the present study, we systematically investigated the clinical significance of tumor-infiltrating (intraepithelial and stromal) lymphocytes in patients using 536 ESCC tissue samples. The survival impacts of various immunological factors were also assessed.

Material and Methods

Study population

Five hundred and thirty-six patients who underwent radical surgery for ESCC between January 2008 and April 2012 in Department of Thoracic Surgery at Zhejiang Cancer Hospital were included in the study. No patients received neoadjuvant treatment or immunotherapy. The demographic and clinical data of patients including age, gender, tumor site, histological grade, lymph node status, pTNM stage, smoking experience, alcohol drinking, and family history were abstracted from clinical records. The extent of the disease was determined by pTNM staging based on the 7th IUCC/AJCC recommendations. All tissue specimens used in our study were obtained from the tissue bank of Zhejiang Cancer Hospital and all patients provided informed consent before surgery. The protocol of the study was approved by the Institutional Review Board of Zhejiang Cancer Hospital.

Follow-up visits were performed every three months for the first two years after surgery, and every six months in the third year and yearly thereafter. At each visit, a clinical history was taken and a physical examination was performed. Routine diagnostic imaging methods included gastroscopy and computer tomography (CT). The median follow-up time was 32.7 months with a range from 1.0 to 88.7 months. The overall survival (OS) data were available for 451 patients (84.1%); among whom 261 patients (57.9%) died during follow-up. Disease free survival (DFS) data were available for 403 patients (75.2%), and 224 patients (41.8%) underwent disease relapse, of which 190 patients (84.8%) died.

Tissue microarray

Formalin-fixed paraffin-embedded (FFPE) surgical specimens were hematoxylin and eosin (H&E) stained, and ESCC was confirmed by two senior pathologists independently. The paraffin tissue blocks of 536 cases of esophageal cancer were used in the construction of the tissue microarray. In brief, the H&E-stained standard slides were reviewed from each section of esophageal cancer tissue, and one representative tumor area of each tumor (2 mm diameter) was removed from the FFPE tissue blocks. Tissue microarray was sectioned at 3 μm and mounted on glass slides for the immunohistochemistry analysis.

Immunohistochemistry analysis

Standard immunohistochemical analysis was performed against primary antibody directed CD3 (clone SP7, diluted at 1: 100, Abcam, Cambridge, UK), CD4 (clone B468A1, diluted at 1: 200, Santa Cruz, Texas, USA) and CD8 (clone 144B, diluted at 1: 100, Abcam, Cambridge, UK). Antigen retrieval was achieved using EDTA buffer (pH 9.0) in a pressure cooker for 20 minutes. After neutralization of endogenous peroxidase, tissue

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

microarray slides were pre-incubated with blocking serum and then were incubated with primary antibody for 45 minutes at room temperature. After three five-minute washes with PBS, the slides were treated with the horseradish peroxidase (HRP)-labeled secondary antibody (Dako, Glostrup, Denmark) for 20 minutes at room temperature, then washed in PBS. Finally, the reaction products were visualized with 3,3'-diaminobenzidine (DAB, Dako, Glostrup, Denmark) and the slides were counterstained with hematoxylin. After being dehydrated, slides were mounted in resin.

Evaluation of immunohistochemistry

The tumor sections were screened at low power magnification (100×), and high power fields (400×) were selected to evaluated the immunoreactivity. All immunostained slides were examined independently by two senior pathologists in a blind manner. The average of the values obtained by the two observers was recorded and used in the statistical analysis. In accordance with previously published approaches [26], intraepithelial TILs (iTILs) were defined as lymphocytes located within tumor cell nests or in direct contact with the ESCC epithelial cells, whereas stromal TILs (sTILs) were defined as lymphocytes in the adjacent peritumoral stroma without direct contact with the carcinoma cells. Areas of necrosis and artifacts, and cells within the vessels were omitted. All samples were scored according to the frequency of positive cells in all nucleated cells (as percentage) on the stained TMA core. The median leukocyte count was used as cutoff to categorize each case into either a high or low group. The tissues having no TILs were excluded from our study; hence data of 514 patients were used to assess the CD3 expression, 530 cases were used to evaluate the CD4 expression, and 527 cases were used for CD8 analysis.

Statistical analysis

Differences between groups were compared using the chi-square test or the Mann-Whitney U test for variables. Spearman's coefficient rank test was used to evaluate the correlation between tumor-infiltrating T lymphocyte, and PD-L1 expression. Associations with DFS and OS were analyzed using the Kaplan-Meier method, log-rank test and the Cox proportional hazards model. All the statistical analyses were performed with SPSS 13.0 for Windows (Chicago, IL, USA), and p value ≤ 0.05 in a two-tailed test was considered statistically significant.

Results

Expression of TIL

The infiltrating T lymphocytes were categorized by location in the tumor intraepithelium (i) or stroma (s), and the representative images of CD3+, CD4+, CD8+ lymphocyte infiltration are shown in Figure 1. The expressions of CD3, CD4, and CD8 were found to be located on both the membrane and in the cytoplasm of TILs. A total of 536 patients were enrolled in this study. Among them, 40.9% (210 patients) had intraepithelial CD3 (iCD3) expression, and 39.1% (199 patients) had stromal CD3 (sCD3) expression, but no significant difference between the infiltration of iCD3+ and sCD3+ lymphocytes was found ($p>0.05$). Regarding CD8 expression in the 527 patients, 16.7% (88 patients) were intraepithelial expression, and 15.4% (81 patients) were stromal expression. Also, the accumulation of iCD8+ lymphocytes was not related to the sCD8+ lymphocytes ($p>0.05$). Importantly, the infiltration of iCD4+ lymphocytes was 44.2% (234/530), which was significantly higher than that in stromal regions (28.9%, 153/530, $p<0.001$).

Clinicopathological associations with TIL expression

The demographics of 536 patients are summarized in Table 1. The median age of the patients at diagnosis was 60 (range 37–77) years. Among these patients, 73.5% (394/536) were younger than 65 years, 86.0% (464/536) were male, 25.7% (138/536) had family history, 71.3% (382/536) were alcohol drinkers, and 75.2% (403/536) were smokers. Within the cohort, 35 patients (6.5%) had well differentiated tumors, 377 (70.3%) moderately differentiated tumors, and 124 (23.1%) poorly differentiated tumors. In accordance with the 7th IUCC/AJCC staging system, 61 (11.4%) patients were stage I, 195 (36.4%) patients were stage II, 273 (50.9%) patients were stage III, and seven (1.3%) patients were stage IV. In the present study, we analyzed the correlations between the clinicopathological features and the infiltration of CD3+, CD4+, and CD8+ lymphocytes in ESCC tissues. As shown in Table 1–6, increased iCD3+, sCD4+, and iCD8+ lymphocytes were significantly associated with advanced tumor differentiation ($p<0.05$). Furthermore, high infiltrations of sCD4+ and sCD8+ lymphocytes were more frequently observed in patients with alcohol drinking and smoking experience ($p<0.05$). However, sCD3+ and iCD4+ lymphocytes had no significant correlation with any of the clinicopathological parameters ($p>0.05$).

Correlations between PD-L1 and TIL expression

Based on the fact that PD-L1 expression had close interactions with TILs in the tumor microenvironment [20], combining our previous study of an inverse association between PD-L1 expression and lymph node metastasis [21], we hypothesized that PD-L1 expression could be induced by increased T-cell infiltration. Then the relevance of PD-L1 expression and various TIL markers was investigated. Unexpectedly, results showed an inverse trend of PD-L1 expression and lymphocytes infiltration, especially in sCD3+ lymphocytes (Table 7, $p=0.027$). Correlation analysis revealed the similar results

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

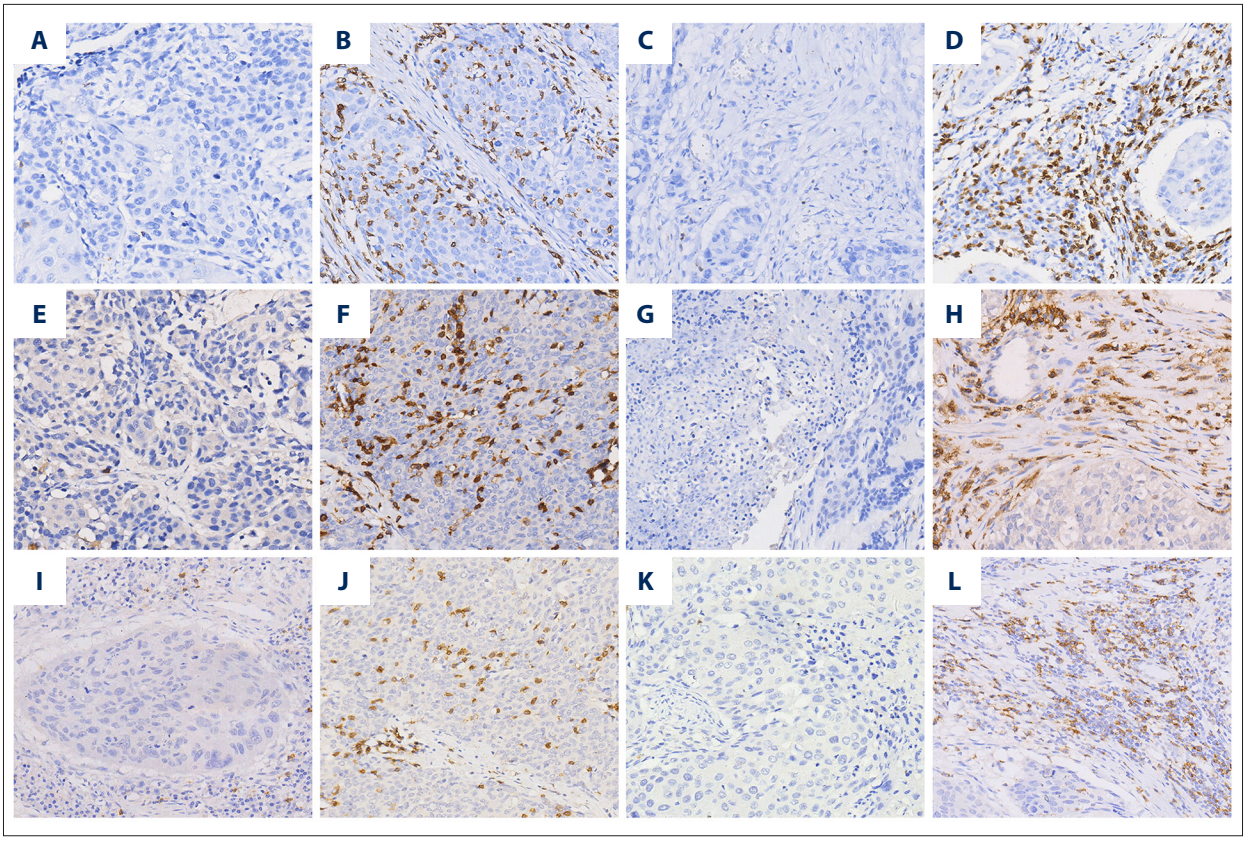

Figure 1. Immunohistochemical staining for human esophageal squamous cell carcinoma (ESCC) tissues recognizing the expressions of CD3 (**top**), CD4 (**middle**) and CD8 (**bottom**) in tumor-infiltrating lymphocytes. Representative cases of: intraepithelial (i) CD3-negative (**A**), iCD3-positive (**B**), stromal (s) CD3-negative (**C**), sCD3-positive (**D**), iCD4-negative (**E**), iCD4-positive (**F**), sCD4-negative (**G**), sCD4-positive (**H**), iCD8-negative (**I**), iCD8-positive (**J**), sCD8-negative (**K**), and sCD8-positive (**L**). Original magnification 200×.

(r=–0.098, p=0.027). Though infiltrated T cells have been reported to induce better outcome in ESCC [23], the patients in our study with PD-L1 expression were more likely to have less T cells infiltration.

Prognostic impacts of the immune microenvironment

To identify variables of potential prognostic significance in the patients with ESCC, the impacts of TILs subgroup and other clinicopathological parameters on the prognosis were explored. Table 7 shows that increased iCD4+ lymphocytes were significantly associated with prolonged overall survival (OS) in ESCC (p=0.007), and the Kaplan-Meier figures are shown in Figure 2. In addition, multivariate analysis implicated that iCD4+ lymphocytes infiltration was an independent predictor for OS after adjusting by clinicopathological factors in ESCC (hazard ratio [HR]=0.67, 95% confidence interval [CI]: 0.51–0.88, p=0.04, Table 8). However, no prognostic significance was found for sCD4+, iCD3+, sCD3+, iCD8+, or sCD8+ lymphocytes in ESCC (p>0.05).

Discussion

In our previous study, we identified that PD-L1 could be a favorable indicator of prognosis in ESCC and patients with its expression were more likely to have less lymph node metastasis [21]. We thus hypothesized the positive prognostic effect of PD-L1 might be induced by increased T-cell infiltration. However, in this study, we detected an inverse association between immune T-cell abundance and PD-L1 expression in ESCC, indicating the interaction of PD-L1 and PD-1 might inhibit the functions of TILs.

Our findings in this study are in accordance with earlier reports about a significantly inverse correlation between PD-L1 expression and infiltrated T lymphocytes [27–29]. In addition, a study of NSCLC elucidated that the PD-L1 protein expression pattern was related to changeable T-cell density within the tumor microenvironment [30]. Therefore, the favorable prognostic effect of PD-L1 might be based on regulatory of the complicated immune network, like the activation of different immune cell subpopulations, like γδ T cells [31],

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 1. Clinicopathological associations with iCD3 expression in ESCC.

Category	All cases		iCD3				*p* Value
			–		+		
Age							
<65	139	(27.0%)	75	(54.0%)	64	(46.0%)	0.145
≥65	375	(73.0%)	229	(61.1%)	146	(38.9%)	
Gender							
Male	444	(86.4%)	265	(59.7%)	179	(40.3%)	0.530
Female	70	(13.6%)	39	(55.7%)	31	(44.3%)	
Tumor site							
Upper	15	(2.9%)	8	(53.3%)	7	(46.7%)	0.784
Middle	146	(28.4%)	84	(57.5%)	62	(42.5%)	
Lower	353	(68.7%)	212	(60.1%)	141	(39.9%)	
Tumor grade							
Well	32	(6.2%)	23	(71.9%)	9	(28.1%)	***0.020***
Moderate	362	(70.4%)	222	(61.3%)	140	(38.7%)	
Poor	120	(23.3%)	59	(49.2%)	61	(50.8%)	
Tumor stage							
I	58	(11.3%)	33	(56.9%)	25	(43.1%)	0.544
II	188	(36.6%)	115	(61.2%)	73	(38.8%)	
III	262	(51.0%)	151	(57.6%)	111	(42.4%)	
IV	6	(1.2%)	5	(83.3%)	1	(16.7%)	
Family history							
Yes	132	(25.7%)	73	(55.3%)	59	(44.7%)	0.298
No	382	(74.3%)	231	(60.5%)	151	(39.5%)	
Alcohol history							
Yes	363	(70.6%)	208	(57.3%)	155	(42.7%)	0.187
No	151	(29.4%)	96	(63.6%)	55	(36.4%)	
Smoking history							
Yes	386	(75.1%)	234	(60.6%)	152	(39.4%)	0.237
No	128	(24.9%)	70	(54.7%)	58	(45.3%)	
BMI							
<18	70	(13.6%)	38	(54.3%)	32	(45.7%)	0.274
18–25	395	(76.8%)	241	(61.0%)	154	(39.0%)	
>25	49	(9.5%)	25	(51.0%)	24	(49.0%)	
Total	514		304	(59.1%)	210	(40.9%)	

Bold-italic value was statistically significant ($p \leq 0.05$).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 2. Clinicopathological associations with sCD3 expression in ESCC.

Category	All cases		sCD3				*p* Value
			–		+		
Age							
<65	139	(27.3%)	82	(59.0%)	57	(41.0%)	0.588
≥65	370	(72.7%)	228	(61.6%)	142	(38.4%)	
Gender							
Male	439	(86.2%)	268	(61.0%)	171	(39.0%)	0.867
Female	70	(13.8%)	42	(60.0%)	28	(40.0%)	
Tumor site							
Upper	15	(2.9%)	7	(46.7%)	8	(53.3%)	0.276
Middle	146	(28.7%)	84	(57.5%)	62	(42.5%)	
Lower	348	(68.4%)	219	(62.9%)	129	(37.1%)	
Tumor grade							
Well	31	(6.1%)	19	(61.3%)	12	(38.7%)	0.653
Moderate	361	(70.9%)	224	(62.0%)	137	(38.0%)	
Poor	117	(23.0%)	67	(57.3%)	50	(42.7%)	
Tumor stage							
I	58	(11.4%)	35	(60.3%)	23	(39.7%)	0.212
II	187	(36.7%)	117	(62.6%)	70	(37.4%)	
III	258	(50.7%)	152	(58.9%)	106	(41.1%)	
IV	6	(1.2%)	6	(100.0%)	0	(0.0%)	
Family history							
Yes	129	(25.3%)	70	(54.3%)	59	(45.7%)	0.074
No	380	(74.7%)	240	(63.2%)	140	(36.8%)	
Alcohol history							
Yes	358	(70.3%)	218	(60.9%)	140	(39.1%)	0.247
No	151	(29.7%)	92	(60.9%)	55	(39.1%)	
Smoking history							
Yes	381	(74.9%)	233	(61.2%)	148	(38.8%)	0.841
No	128	(25.1%)	77	(60.2%)	51	(39.8%)	
BMI							
<18	70	(13.8%)	40	(57.1%)	30	(42.9%)	0.176
18–25	391	(76.8%)	246	(62.9%)	145	(37.1)	
>25	48	(9.4%)	24	(50.0%)	24	(50.0%)	
Total	509		310	(60.9%)	199	(39.1%)	

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 3. Clinicopathological associations with iCD4 expression in ESCC.

Category	All cases		iCD4				*p* Value
			–		+		
Age							
<65	141	(26.6%)	73	(51.8%)	68	(48.2%)	0.255
≥65	389	(73.4%)	223	(57.3%)	166	(42.7%)	
Gender							
Male	459	(86.6%)	260	(56.6%)	199	(43.4%)	0.348
Female	71	(13.4%)	36	(50.7%)	35	(49.3%)	
Tumor site							
Upper	16	(3.0%)	7	(43.8%)	9	(56.3%)	0.586
Middle	152	(28.7%)	87	(57.2%)	65	(42.8%)	
Lower	362	(68.3%)	202	(55.8%)	160	(44.2%)	
Tumor grade							
Well	35	(6.6%)	18	(51.4%)	17	(48.6%)	0.784
Moderate	372	(70.2%)	211	(56.7%)	161	(43.3%)	
Poor	123	(23.2%)	67	(54.5%)	56	(45.5%)	
Tumor stage							
I	59	(11.1%)	31	(52.5%)	28	(47.5%)	0.264
II	193	(36.4%)	102	(52.8%)	91	(47.2%)	
III	271	(51.1%)	157	(57.9%)	114	(42.1%)	
IV	7	(1.3%)	6	(85.7%)	1	(14.3%)	
Family history							
Yes	134	(25.3%)	76	(56.7%)	58	(43.3%)	0.815
No	396	(74.7%)	220	(55.6%)	176	(44.4%)	
Alcohol history							
Yes	377	(71.1%)	212	(56.2%)	165	(43.8%)	0.780
No	153	(28.9%)	84	(54.9%)	69	(45.1%)	
Smoking history							
Yes	399	(75.3%)	225	(56.4%)	174	(43.6%)	0.661
No	131	(24.7%)	71	(54.2%)	60	(45.8%)	
BMI							
<18	71	(13.4%)	37	(52.1%)	34	(47.9%)	0.733
18–25	407	(76.8%)	231	(56.8%)	176	(43.2%)	
>25	52	(9.8%)	28	(53.8%)	24	(46.2%)	
Total	530		296	(55.8%)	234	(44.2%)	

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 4. Clinicopathological associations with sCD4 expression in ESCC.

Category	All cases		sCD4				*p* Value
			–		+		
Age							
<65	141	(26.6%)	96	(68.1%)	45	(31.9%)	0.351
≥65	389	(73.4%)	281	(72.2%)	108	(27.8%)	
Gender							
Male	459	(86.6%)	323	(70.4%)	136	(29.6%)	0.325
Female	71	(13.4%)	54	(76.1%)	17	(23.9%)	
Tumor site							
Upper	16	(3.0%)	13	(81.3%)	3	(18.7%)	0.264
Middle	152	(28.7%)	114	(75.0%)	38	(25.0%)	
Lower	362	(68.3%)	250	(69.1%)	112	(30.9%)	
Tumor grade							
Well	35	(6.6%)	27	(77.1%)	8	(22.9%)	***0.017***
Moderate	372	(70.2%)	275	(73.9%)	97	(26.1%)	
Poor	123	(23.2%)	75	(61.0%)	48	(39.0%)	
Tumor stage							
I	59	(11.1%)	40	(67.8%)	19	(32.2%)	0.323
II	193	(36.4%)	140	(72.5%)	53	(27.5%)	
III	271	(51.1%)	190	(70.1%)	81	(29.9%)	
IV	7	(1.3%)	7	(100.0%)	0	(0.0%)	
Family history							
Yes	134	(25.3%)	95	(70.9%)	39	(29.1%)	0.944
No	396	(74.7%)	282	(71.2%)	114	(28.8%)	
Alcohol history							
Yes	377	(71.1%)	257	(68.2%)	120	(31.8%)	***0.018***
No	153	(28.9%)	120	(78.4%)	33	(21.6%)	
Smoking history							
Yes	399	(75.3%)	275	(68.9%)	124	(31.1%)	***0.050***
No	131	(24.7%)	102	(77.9%)	29	(22.1%)	
BMI							
<18	71	(13.4%)	48	(67.6%)	23	(32.4%)	0.527
18–25	407	(76.8%)	289	(71.0%)	118	(29.0%)	
>25	52	(9.8%)	40	(76.9%)	12	(423.1%)	
Total	530		377	(71.1%)	153	(28.9%)	

Bold-italic value was statistically significant ($p \leq 0.05$).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 5. Clinicopathological associations with iCD8 expression in ESCC.

Category	All cases		iCD8				*p* Value
			–		+		
Age							
<65	140	(26.6%)	117	(83.6%)	23	(16.4%)	0.920
≥65	387	(73.4%)	322	(83.2%)	65	(16.8%)	
Gender							
Male	458	(86.9%)	385	(84.1%)	73	(15.9%)	0.228
Female	69	(13.1%)	54	(78.3%)	15	(21.7%)	
Tumor site							
Upper	16	(3.0%)	14	(87.5%)	2	(12.5%)	0.608
Middle	151	(28.7%)	129	(85.4%)	22	(14.6%)	
Lower	360	(68.3%)	296	(82.2%)	64	(17.8%)	
Tumor grade							
Well	34	(6.5%)	34	(100.0%)	0	(0.0%)	***0.004***
Moderate	370	(70.2%)	311	(84.1%)	59	(15.9%)	
Poor	123	(23.3%)	94	(76.4%)	29	(23.6%)	
Tumor stage							
I	59	(11.2%)	50	(84.7%)	9	(15.3%)	0.958
II	193	(36.6%)	162	(83.9%)	31	(36.1%)	
III	268	(50.9%)	221	(82.5%)	47	(17.5%)	
IV	7	(1.3%)	6	(85.7%)	1	(14.3%)	
Family history							
Yes	133	(25.2%)	112	(84.2%)	21	(15.8%)	0.745
No	394	(74.8%)	327	(83.0%)	67	(17.0%)	
Alcohol history							
Yes	376	(71.3%)	310	(82.4%)	66	(17.6%)	0.406
No	151	(28.7%)	129	(85.4%)	22	(14.6%)	
Smoking history							
Yes	397	(75.3%)	332	(83.6%)	65	(16.4%)	0.726
No	130	(24.7%)	107	(82.3%)	23	(17.7%)	
BMI							
<18	71	(13.5%)	55	(54.3%)	16	(45.7%)	0.365
18–25	404	(76.7%)	340	(61.0%)	64	(39.0%)	
>25	52	(9.9%)	44	(51.0%)	8	(49.0%)	
Total	527		439	(83.3%)	88	(16.7%)	

Bold-italic value was statistically significant ($p \leq 0.05$).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 6. Clinicopathological associations with sCD8 expression in ESCC.

Category	All cases		sCD8				*p* Value
			–		+		
Age							
<65	140	(26.6%)	120	(85.7%)	20	(14.3%)	0.678
≥65	387	(73.4%)	326	(84.2%)	61	(15.8%)	
Gender							
Male	458	(86.9%)	384	(83.8%)	74	(16.2%)	0.197
Female	69	(13.1%)	62	(89.9%)	7	(10.1%)	
Tumor site							
Upper	16	(3.0%)	13	(81.3%)	3	(18.8%)	0.665
Middle	151	(28.7%)	131	(86.8%)	20	(13.2%)	
Lower	360	(68.3%)	302	(83.9%)	58	(16.1%)	
Tumor grade							
Well	123	(23.3%)	104	(84.6%)	19	(15.4%)	0.832
Moderate	370	(70.2%)	312	(84.3%)	58	(15.7%)	
Poor	34	(6.5%)	30	(88.2%)	4	(11.8%)	
Tumor stage							
I	59	(11.2%)	49	(83.1%)	10	(16.9%)	0.701
II	193	(36.6%)	164	(85.0%)	29	(15.0%)	
III	268	(50.9%)	226	(84.3%)	42	(15.7%)	
IV	7	(1.3%)	7	(1.6%)	0	(0.0%)	
Family history							
Yes	133	(25.2%)	113	(85.0%)	20	(15.0%)	0.902
No	394	(74.8%)	333	(84.5%)	61	(15.5%)	
Alcohol history							
Yes	376	(71.3%)	309	(82.3%)	67	(17.8%)	***0.014***
No	151	(28.7%)	137	(90.7%)	14	(9.3%)	
Smoking history							
Yes	397	(75.3%)	328	(82.6%)	69	(17.4%)	***0.025***
No	130	(24.7%)	118	(90.8%)	12	(9.2%)	
BMI							
<18	71	(13.5%)	61	(85.9%)	10	(14.1%)	0.658
18–25	404	(76.7%)	339	(83.9%)	65	(16.1%)	
>25	52	(9.9%)	46	(88.5%)	6	(11.5%)	
Total	527		446	(84.6%)	81	(15.4%)	

Bold-italic value was statistically significant ($p \leq 0.05$).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 7. The associations of T cells infiltration with programmed death-ligand 1 (PD-L1) expression and the survival of esophageal squamous cell carcinoma patients.

Variables	NO. of patients (%)	PD-L1 expression		p Value	HR for DFS	p Value	HR for OS	p Value
		Positive (%)	Negative (%)		(95% CI)		(95% CI)	
Intraepithelium								
CD3+T cell								
Positive	210 (40.9)	81 (38.6)	129 (61.4)	0.143	1.008	0.978	0.954	0.847
Negative	304 (59.1)	137 (45.1)	167 (54.9)		(0.596–1.702)		(0.589–1.544)	
CD4+T cell								
Positive	234 (44.2)	98 (41.9)	136 (58.1)	0.940	0.664	0.130	0.517	***0.007***
Negative	296 (55.8)	123 (41.6)	173 (58.4)		(0.392–1.127)		(0.319–0.838)	
CD8+T cell								
Positive	88 (16.7)	29 (33.0)	59 (67.0)	0.079	0.731	0.325	0.943	0.841
Negative	439 (83.3)	189 (43.1)	250 (56.9)		(0.391–1.365)		(0.533–1.670)	
Stroma								
CD3+T cell								
Positive	199 (39.1)	72 (36.2)	127 (63.8)	***0.027***	0.935	0.771	1.061	0.802
Negative	310 (60.9)	143 (46.1)	167 (53.9)		(0.594–1.472)		(0.670–1.680)	
CD4+T cell								
Positive	153 (28.9)	60 (39.2)	93 (60.8)	0.460	0.431	0.418	0.398	0.373
Negative	377 (71.1)	161 (42.7)	216 (57.3)		(0.056–3.307)		(0.052–3.029)	
CD8+T cell								
Positive	81 (15.4)	31 (38.3)	50 (61.7)	0.539	0.873	0.597	0.651	0.071
Negative	446 (84.6)	187 (41.9)	259 (58.1)		(0.528–1.444)		(0.408–1.037)	

Bold-italic values were statistically significant ($p \leq 0.05$); HR – hazard ratio; CI – confidence interval.

and the secretion of certain cytokines such as IL-10 and interferon γ [32–34]. Meanwhile, the prognostic significance of PD-L1 has been proven to be relevant to the mutational burden [35], inducing increased presentation of neo-antigens by tumor cells [17]. However, other studies about the presence of TILs in human tumor systems including ESCC have showed a positive or no association with PD-L1 expression [33,36–38]. Taken together, the regulatory effect of the complicated immune network in the tumor microenvironment has not been fully determined until now.

In the present study, we also explored the impact of tumor-infiltrating lymphocytes on the clinical significance in ESCC. The cooperation of CD4+ and CD8+ T cells has been well-documented in host immune responses against various tumors including ESCC [22,23]. In our study, the total number of CD4+ TILs in intraepithelium was markedly higher than those in stromal areas. Consistent with our study, Hatogai et al. also found intraepithelial TILs had significantly increased levels compared to stromal compartments, conferring more clinical prognostic value in ESCC [38].

In line with previous reports in ovarian carcinoma [39], we observed that patients with increased infiltration of iCD4+ lymphocytes had longer OS compared to those with decreased infiltration, while sCD4+ lymphocytes was not correlated with the outcomes in ESCC. The localization of TILs seems have major relevance with regards to their prognostic impact; this finding should encourage highly interesting research in the future. Furthermore, subpopulations of CD4+ T cells could have different functional impacts on the immune environment; for instance CD4+ T cells can have anti-tumorigenic effects when the

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

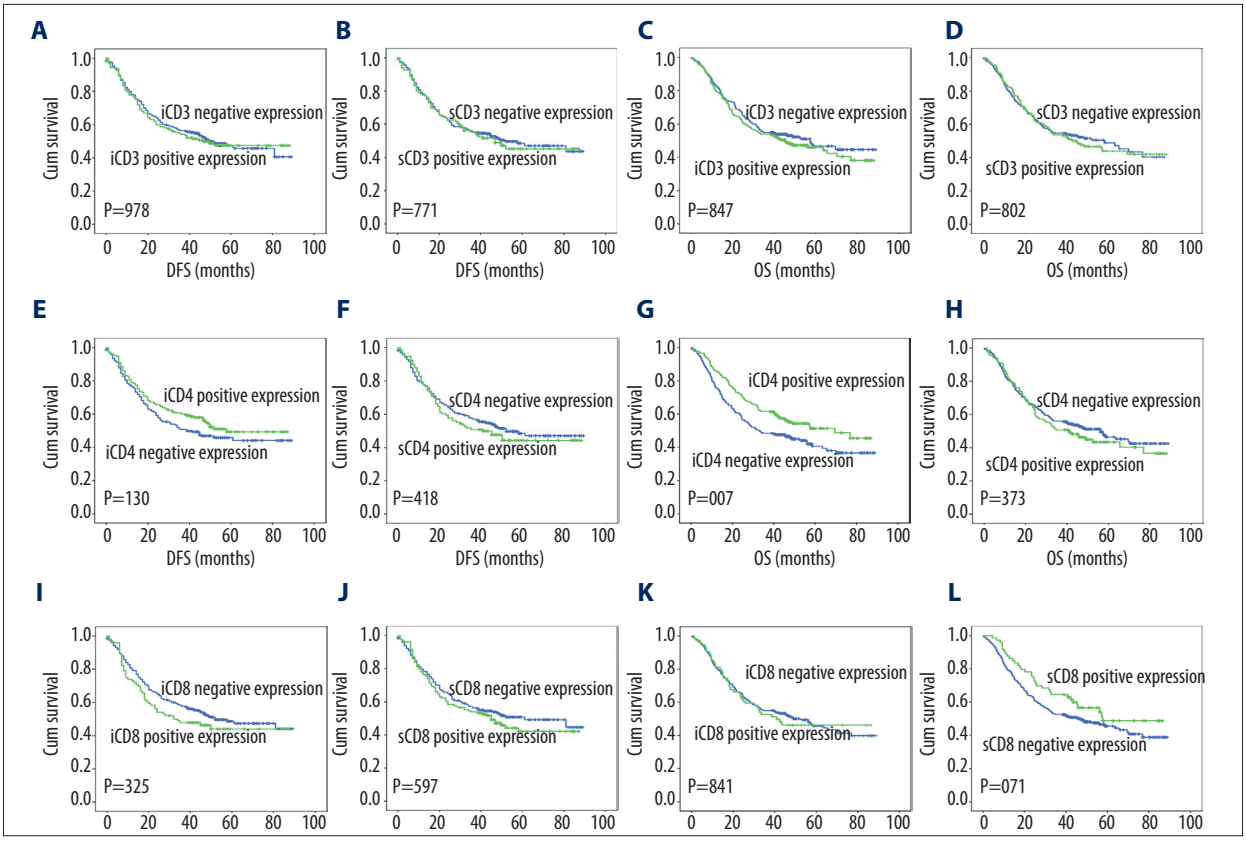

Figure 2. Kaplan-Meier curves of disease-free survival (DFS) and overall survival (OS) in esophageal squamous cell carcinoma (ESCC) based on tumor-infiltrating lymphocytes. (**A–D**), increased iCD3+ or sCD3+ lymphocytes infiltration was not associated with DFS and OS in ESCC (p>0.05); (**E–H**), increased iCD4+ lymphocytes were significantly associated with longer OS in ESCC (p=0.007); (**I–L**), no prognostic significance was found for iCD8+ or sCD8+ lymphocytes in ESCC (p>0.05).

Th1 subset is active in secreting pro-inflammatory cytokines, whereas a pro-tumorigenic effect can be predominant when Th2 cells secrete anti-inflammatory cytokines [40]. However, in our study, no correlations between the infiltration of iCD3+ lymphocytes and sCD3+ lymphocytes and their impacts on the prognosis were found. And iCD8+ and sCD8+ lymphocytes infiltration was also not associated with clinical outcome in ESCC. Notably, one study found increased CD8+ TILs infiltration were independent favorable prognostic factors in esophageal adenocarcinoma [41], while another study indicated that the TILs were not the prognostic markers for long-term survival in patients with esophageal adenocarcinoma [42]. These controversial results were likely caused by factors in the tissue microenvironment which may alter T-cell phenotype and function early during esophageal disease progression and may represent targets for immune intervention [43]. Further investigations regarding the impact on clinical outcomes of the balance of immune infiltrating cells are needed.

Additionally, the presence of stromal TILs infiltration more than intraepithelial infiltration was correlated with alcohol drinking and smoking experience. As previously described, smoking history of a patient was associated with a greater risk of cancer development than patients without those characteristics, and were characterized by abundant and deregulated inflammation [44], suggesting a linkage between inflammatory processes affecting the smokers or alcohol drinkers and immune response status of tumors [30]. The selective recruitment of T cells to the stromal compartment of these esophageal cancers suggests that these stromal TILs are less affected by tumor-derived inhibitory factors than in the intraepithelial regions, which are located in direct contact with malignant cells [30]. Unlike an earlier study [22], we observed patients with increased TILs were more likely to harbor poor tumor differentiation; meanwhile, some other authors reported no association of TILs accumulation with tumor grade of ESCC [23,38]. Potential explanations for this finding might be induced from a misbalance of the effect of tumor-derived factors and immune responses.

Based on a large cohort of operable ESCC patients, our study systematically described the immune status in the tumor microenvironment. To our knowledge, this is the first study that has indicated that increased iCD4+ lymphocytes infiltration was

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 8. Prognostic factors for disease free survival and overall survival of operable ESCC patients estimated by multivariate Cox regression analyses.

Parameters	DFS		OS	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Intraepithelial CD3+ T cell				
Negative	1.00		1.00	
Positive	1.01 (0.60–1.70)	0.978	1.26 (0.76–2.11)	0.371
Stromal CD3+ T cell				
Negative	1.00		1.00	
Positive	0.94 (0.60–1.47)	0.771	1.17 (0.78–1.75)	0.442
Intraepithelial CD4+ T cell				
Negative	1.00		1.00	
Positive	0.66 (0.40–1.13)	0.130	0.67 (0.51–0.88)	***0.004***
Stromal CD4+ T cell				
Negative	1.00		1.00	
Positive	0.43 (0.06–3.31)	0.418	0.41 (0.05–3.31)	0.386
Intraepithelial CD8+ T cell				
Negative	1.00		1.00	
Positive	0.73 (0.39–1.37)	0.325	0.89 (0.50–1.58)	0.325
Stromal CD8+ T cell				
Negative	1.00		1.00	
Positive	0.87 (0.53–1.44)	0.597	0.65 (0.40–1.04)	0.075

Bold-italic value was statistically significant ($p \leq 0.05$); HR – hazard ratio; CI – confidence interval; Adjusting for age, sex, tumor site, stage, grade, smoking experience, PD-L1 expression, alcohol drinking and family history.

an independent favorable predictor for prognosis in ESCC. In addition, this study has its own limitations. Our data did not include the responses rate of anti-PD-1 and anti-PD-L1 therapeutics in ESCC patients; and functional studies to validate and understand the roles of TILs and cytokines such as interferon γ are urgently needed.

Conclusions

The iCD4+ lymphocytes infiltration is an independent favorable factor for prognosis in ESCC. The TILs infiltration in ESCC tissues was inversely associated with the expression of PD-L1. Therefore, the profiles of immune cells may provide additional information for patient outcomes and help to stratify patients who will benefit more from immunotherapy.

References:

1. Chen W, Zheng R, Baade PD et al: Cancer statistics in China, 2015. Cancer J Clin, 2016; 66(2): 115–32
2. Song Y, Li L, Ou Y et al: Identification of genomic alterations in oesophageal squamous cell cancer. Nature, 2014; 509(7498): 91–95
3. Xu Y, Yu X, Chen Q, Mao W: Neoadjuvant versus adjuvant treatment: which one is better for resectable esophageal squamous cell carcinoma? World J Surg Oncol, 2012; 10: 173
4. Kleinberg L, Forastiere AA: Chemoradiation in the management of esophageal cancer. J Clin Oncol, 2007; 25(26): 4110–17
5. Han X, Lu N, Pan Y, Xu J: Nimotuzumab combined with chemotherapy is a promising treatment for locally advanced and metastatic esophageal cancer. Med Sci Monit, 2017; 23: 412–18
6. Pardoll DM: The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer, 2012; 12(4): 252–64
7. Reiss KA, Forde PM, Brahmer JR: Harnessing the power of the immune system via blockade of PD-1 and PD-L1: A promising new anticancer strategy. Immunotherapy, 2014; 6(4): 459–75

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

8. Anagnostou VK, Brahmer JR: Cancer immunotherapy: A future paradigm shift in the treatment of non-small cell lung cancer. Clin Cancer Res, 2015; 21(5): 976–84
9. Topalian SL, Hodi FS, Brahmer JR et al: Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med, 2012; 366(26): 2443–54
10. Daskivich TJ, Belldegrun A: Words of wisdom. Re: Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. Eur Urol, 2015; 67(4): 816–17
11. Brahmer JR, Tykodi SS, Chow LQ et al: Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med, 2012; 366(26): 2455–65
12. Baptista MZ, Sarian LO, Derchain SF et al: Prognostic significance of PD-L1 and PD-L2 in breast cancer. Hum Pathol, 2016; 47(1): 78–84
13. Thierauf J, Veit JA, Affolter A et al: Identification and clinical relevance of PD-L1 expression in primary mucosal malignant melanoma of the head and neck. Melanoma Res, 2015; 25(6): 503–9
14. Liu Y, Carlsson R, Ambjorn M et al: PD-L1 expression by neurons nearby tumors indicates better prognosis in glioblastoma patients. J Neurosci, 2013; 33(35): 14231–45
15. Darb-Esfahani S, Kunze CA, Kulbe H et al: Prognostic impact of programmed cell death-1 (PD-1) and PD-ligand 1 (PD-L1) expression in cancer cells and tumor-infiltrating lymphocytes in ovarian high grade serous carcinoma. Oncotarget, 2016; 7(2): 1486–99
16. Yang CY, Lin MW, Chang YL et al: Programmed cell death-ligand 1 expression is associated with a favourable immune microenvironment and better overall survival in stage I pulmonary squamous cell carcinoma. Eur J Cancer, 2016; 57: 91–103
17. Rizvi NA, Hellmann MD, Snyder A et al: Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science, 2015; 348(6230): 124–28
18. Sui X, Ma J, Han W et al: The anticancer immune response of anti-PD-1/PD-L1 and the genetic determinants of response to anti-PD-1/PD-L1 antibodies in cancer patients. Oncotarget, 2015; 6(23): 19393–404
19. Xu F, Xu L, Wang Q et al: Clinicopathological and prognostic value of programmed death ligand-1 (PD-L1) in renal cell carcinoma: A meta-analysis. Int J Clin Exp Med, 2015; 8(9): 14595–603
20. Droeser RA, Hirt C, Viehl CT et al: Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. Eur J Cancer, 2013; 49(9): 2233–42
21. Chen K, Cheng G, Zhang F et al: Prognostic significance of programmed death-1 and programmed death-ligand 1 expression in patients with esophageal squamous cell carcinoma. Oncotarget, 2016; 7(21): 30772–80
22. Schumacher K, Haensch W, Roefzaad C, Schlag PM: Prognostic significance of activated CD8(+) T cell infiltrations within esophageal carcinomas. Cancer Res, 2001; 61(10): 3932–36
23. Cho Y, Miyamoto M, Kato K et al: CD4+ and CD8+ T cells cooperate to improve prognosis of patients with esophageal squamous cell carcinoma. Cancer Res, 2003; 63(7): 1555–59
24. Ostrand-Rosenberg S, Horn LA, Haile ST: The programmed death-1 immune-suppressive pathway: Barrier to antitumor immunity. J Immunol, 2014; 193(8): 3835–41
25. Kondo A, Yamashita T, Tamura H et al: Interferon-gamma and tumor necrosis factor-alpha induce an immunoinhibitory molecule, B7-H1, via nuclear factor-kappaB activation in blasts in myelodysplastic syndromes. Blood, 2010; 116(7): 1124–31
26. Wang J, Jia Y, Wang N et al: The clinical significance of tumor-infiltrating neutrophils and neutrophil-to-CD8+ lymphocyte ratio in patients with resectable esophageal squamous cell carcinoma. J Transl Med, 2014; 12: 7
27. Hamanishi J, Mandai M, Iwasaki M et al: Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci USA, 2007; 104(9): 3360–65
28. Karunarathne DS, Horne-Debets JM, Huang JX et al: Programmed death-1 ligand 2-mediated regulation of the PD-L1 to PD-1 axis is essential for establishing CD4(+) T cell immunity. Immunity, 2016; 45(2): 333–45
29. Dong H, Strome SE, Salomao DR et al: Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med, 2002; 8(8): 793–800
30. Parra ER, Behrens C, Rodriguez-Canales J et al: Image analysis-based assessment of PD-L1 and tumor-associated immune cells density supports distinct intratumoral microenvironment groups in non-small cell lung carcinoma patients. Clin Cancer Res, 2016; 22(24): 6278–89
31. Iwasaki M, Tanaka Y, Kobayashi H et al: Expression and function of PD-1 in human gammadelta T cells that recognize phosphoantigens. Eur J Immunol, 2011; 41(2): 345–55
32. Kinter AL, Godbout EJ, McNally JP et al: The common gamma-chain cytokines IL-2, IL-7, IL-15, and IL-21 induce the expression of programmed death-1 and its ligands. J Immunol, 2008; 181(10): 6738–46
33. Ohigashi Y, Sho M, Yamada Y et al: Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res, 2005; 11(8): 2947–53
34. Wang S, Bajorath J, Flies DB et al: Molecular modeling and functional mapping of B7-H1 and B7-DC uncouple costimulatory function from PD-1 interaction. J Exp Med, 2003; 197(9): 1083–91
35. Le DT, Uram JN, Wang H et al: PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med, 2015; 372(26): 2509–20
36. Lipson EJ, Vincent JG, Loyo M et al: PD-L1 expression in the Merkel cell carcinoma microenvironment: association with inflammation, Merkel cell polyomavirus and overall survival. Cancer Immunol Res, 2013; 1(1): 54–63
37. Kim MY, Koh J, Kim S et al: Clinicopathological analysis of PD-L1 and PD-L2 expression in pulmonary squamous cell carcinoma: Comparison with tumor-infiltrating T cells and the status of oncogenic drivers. Lung Cancer, 2015; 88(1): 24–33
38. Hatogai K, Kitano S, Fujii S et al: Comprehensive immunohistochemical analysis of tumor microenvironment immune status in esophageal squamous cell carcinoma. Oncotarget, 2016; 7(30): 47252–64
39. Clarke B, Tinker AV, Lee CH et al: Intraepithelial T cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss. Mod Pathol, 2009; 22(3): 393–402
40. Crotty S: Follicular helper CD4 T cells (TFH). Annu Rev Immunol, 2011; 29: 621–63
41. Noble F, Mellows T, McCormick Matthews LH et al: Tumour infiltrating lymphocytes correlate with improved survival in patients with oesophageal adenocarcinoma. Cancer Immunol Immunother, 2016; 65(6): 651–62
42. Zingg U, Montani M, Frey DM et al: Tumour-infiltrating lymphocytes and survival in patients with adenocarcinoma of the oesophagus. Eur J Surg Oncol, 2010; 36(7): 670–77
43. Kavanagh ME, Conroy MJ, Clarke NE et al: Impact of the inflammatory microenvironment on T-cell phenotype in the progression from reflux oesophagitis to Barrett oesophagus and oesophageal adenocarcinoma. Cancer Lett, 2016; 370(1): 117–24
44. Chung KF, Adcock IM: Multifaceted mechanisms in COPD: Inflammation, immunity, and tissue repair and destruction. Eur Respir J, 2008; 31(6): 1334–56

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]