Received: 24 August 2022 | Accepted: 21 September 2022

DOI: 10.1111/1759-7714.14683

ORIGINAL ARTICLE

WILEY

Impact of the combination of sintilimab and chemotherapy on the tumor and paratumor PD-L1, IDO, TIM-3, FOXP3+ and CD8 expressions in patients with advanced esophageal squamous cell carcinoma

Shifa Zhang[1,2] | **Haibo Cai**[2] | **Junjun Huang**[3] | **Gongchao Wang**[1]

[1]Department of thoracic surgery, Shandong Provincial Hospital, Shandong University, Jinan, Shandong, China

[2]Department of thoracic surgery, Jining NO.1 People's Hospital, Affiliated Jining NO.1 people's Hospital of Jining Medical University, Shandong, China

[3]Department of Clinical Medicine, Jining Medical University, Jining, China

Correspondence
Gongchao Wang, Department of Thoracic Surgery, Shandong Provincial Hospital, Shandong University, No.9677 Jingshi Road, Jinan, Shandong, 250012, China.
Email: wanggongchao@126.com

Abstract

Background: Anti-PD-1/PD-L1 therapeutics have been widely used in the clinic in various tumors, including advanced esophageal cancer, showing remarkable treatment efficacy. Factors determining the response to anti-PD-1/PD-L1 therapeutics are numerous, including the tumor microenvironment, such as CD8+ T cells and expression of PD-1/PD-L1. Our study aimed to explore the effect of chemoimmunotherapy on the expression of CD8+ T cells, TIM-3, and FOXP3+ in tumor, paratumor tissues, and the expression of PD-L1, IDO, in tumor, paratumor tissues, and lymph nodes, and analyze the correlation among these markers.

Methods: A total of 18 patients were allocated into two treatment groups: a treatment group and a concurrent control group. A total of 38 tissue samples, 114 slides (tumor, paratumor, and lymph node) were collected in the treatment group, and 37 tissue samples, 111 slides (tumor, paratumor, and lymph node) were collected in the concurrent control group.

Results: The expression of PD-L1, CD8+, FOXP3+, TIM-3, and IDO in tumors, paratumor tissues, but not lymph nodes, was significantly affected by chemoimmunotherapy. Compared with patients without chemoimmunotherapy, the expression of CD8+ T cells, IDO, and PD-L1 was significantly decreased in tumor and paratumor tissues after chemoimmunotherapy, while FOXP3+ expression was significantly decreased only in tumor tissues, and TIM-3 expression was significantly decreased only in paratumor tissues. Moreover, the correlation between these markers was also completely altered after chemoimmunotherapy. In addition, N staging was associated with high expression of CD8 in advanced esophageal squamous cell carcinoma in the concurrent control group.

Conclusion: This study provides new insight into the effects of CI treatment on isolated CD8+ T cell infiltration, PD-L1, IDO, FOXP3+ and TIM-3 expression as well as their cross-talk in different tissues enabling a better understanding of the impact of CI treatment on the immune microenvironment.

KEYWORDS
esophageal cancer, IDO, immunotherapy, PD-1 inhibitors, PD-L1, TIM-3

INTRODUCTION

In China, esophageal squamous cell carcinoma (ESCC) is the predominant subtype and accounts for >90% of all

Shifa Zhang, Haibo Cai and Junjun Huang contributed equally to this study.

esophageal cancer cases.[1,2] As patients with ESCC are often clinically diagnosed at the middle and advanced stages, surgery alone may not be a good option. Neoadjuvant therapy before surgery is necessary to facilitate R0 resection and reduce local recurrence.[3]

Chemotherapy has been widely used in preoperative neoadjuvant therapy for esophageal cancer. However, chemotherapy alone has shown limited effects,[4,5] often combined with radiotherapy or other regimens. With the increasingly applied immunotherapy in the treatment of esophageal cancer, neoadjuvant chemoimmunotherapy has become a promising treatment for patients with advanced esophageal squamous cell carcinoma. Several studies have demonstrated that immunotherapy combined with chemotherapy improves the objective response rate (ORR) and survival in multiple tumors and that treatment-related adverse events (TRAEs) are well tolerated.[6–8] Chemotherapy significantly triggers the tumor microenvironment regulation and achieves a synergic effect with immunotherapy.[9] However, the biomarkers of this synergic effect and how they influenced the antitumor immune were not fully understood.

To our knowledge, neoadjuvant chemotherapy has been shown to increase programmed death-1-ligand 1 (PD-L1) expression and CD8+ tumor infiltrating lymphocytes (TILs) in ESCC,[10] and a study has shown that T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) expression is upregulated in response to PD-1 blockade, both in vitro and in vivo.[11] Moreover, indoleamine 2,3-dioxygenase (IDO) and interferon gamma (IFN-γ) are highly expressed after treatment with PD-1 or PD-L1 inhibitors.[12] In addition, previous studies have demonstrated that these markers, alone or together, could be used to determine whether to deliver immunotherapy and evaluate the response to therapy. For instance, CD8+ is currently a reliable biomarker for predicting anti-PD-1 drug therapy in many tumors, although its predictive role in esophageal squamous cell carcinoma is still unclear.[13] The expression of PD-L1 was significantly higher in responders to anti-PD1 therapy than in nonresponders.[14,15] IIDO, which not only can suppress CD8+ T cells but also enhance the activation of regulatory cells (Treg) and myeloid-derived suppressor cells (MDSCs), may be involved in resistance to chemotherapy and anti-PD-1 therapy.[16,17] TIM-3 has been reported to determine the exhaustion and dysfunction of CD8+ TILs and CD4+ by binding to galectin-9, phosphatidylserine (PtdSer), high mobility group protein B1 (HMGB1),[18–22] and carcinoembryonic antigen cell adhesion molecule 1 (CEASAM-1). Yang et al. reported that galectin-9 promotes TIM-3+ T cell apoptosis by crosslinking TIM-3, coexpressed PD-1 attenuates Gal-9/TIM-3-induced apoptosis by promoting the formation of TIM-3/Gal-9/PD-1 lattices.[23] The above cells and molecules in the tumor microenvironment (TME) were all interrelated, triggering synergistic and antagonistic associations (Figure 1). Therefore, we could not accurately predict the change in the TME after chemoimmunotherapy (CI) using a single marker, and a combination of these markers was needed.

Thus, we planned this study to investigate the status of the tumor microenvironment with or without chemoimmunotherapy by the expression changes of CD8+, PD-L1, IDO, TIM-3, forkhead box P3 (FOXP3+) in patients with advanced esophageal squamous cell carcinoma. Meanwhile, we also examined the differential expression of these markers in diverse tissues, such as tumors, paratumors, and

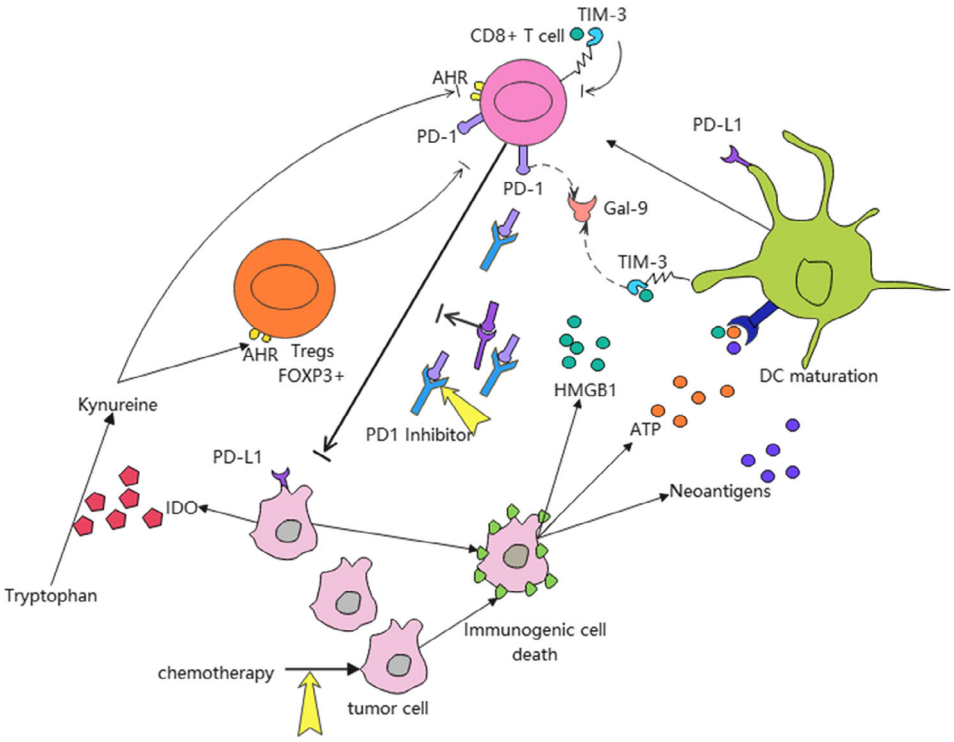

FIGURE 1 Diagram of these indicators. The yellow arrows indicate the chemoimmunotherapy intervention point. AHR, aryl hydrocarbon receptor; ATP, adenosine triphosphate; DC, dendritic cell; FOXP3+, forkhead box P3; Gal-9, galectin-9; HMGB1, high mobility group protein. B1; IDO, indoleamine 2,3-dioxygenase; PD-L1, programmed death ligand 1; PtdSer, phosphatidylserine; TIM-3, T cell immunoglobulin and mucin domain-containing protein 3; Treg, T regulatory cells.

lymph nodes. In addition, we analyzed the correlation among these markers and the correlation between these markers and clinical characteristics.

METHODS

Patients and tissue sample collection

A total of 18 patients were allocated into two groups: a treatment group and a concurrent control group. A total of 38 tissue samples, 114 slides (subgroup: treatment tumor, subgroup: treatment paratumor, and subgroup: treatment lymph node) were collected from March 2020 to February 2022 for the treatment group; and 37 tissue samples, 111 slides (subgroup: control tumor, subgroup: control paratumor and subgroup: control lymph node) were collected from March 2020 to February 2022 for the concurrent control group (Figure 2). For the treatment group, patients with ESCC (stage IIA to IIIB) received anti-PD-1 immunotherapy with sintilimab (200 mg every 3 weeks), cisplatin 40 mg day1–3, and paclitaxel for injection (albumin bound) 300 mg day one were collected in Jining No.1 People's hospital. The concurrent control group patients underwent surgery without any preoperative treatment. Tissue samples, including tumor, paratumor, and lymph node were collected immediately after surgery and patients underwent surgery 4 weeks after 2–3 cycles of chemoimmunotherapy. The

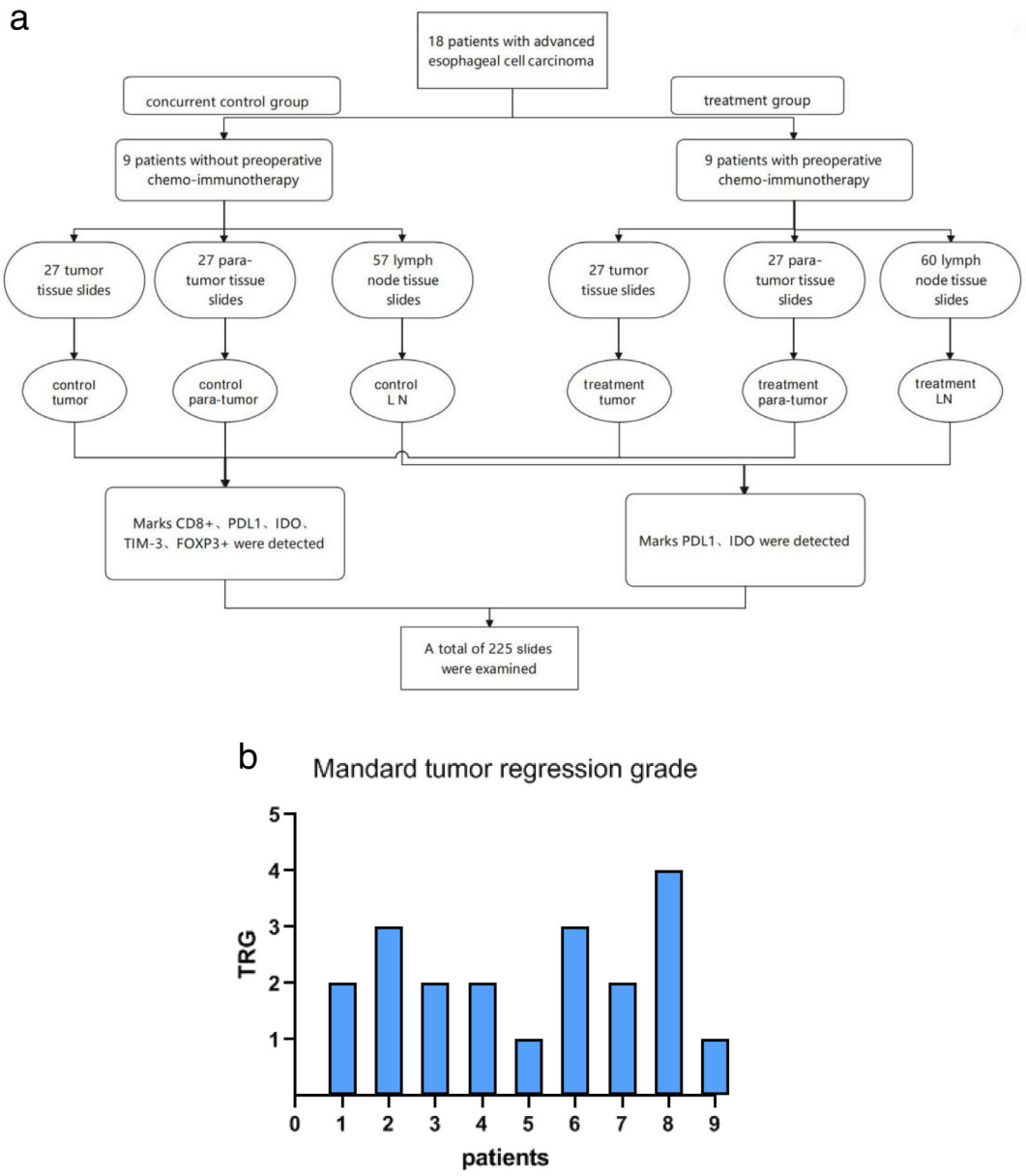

FIGURE 2 (a) Flow chart of the experiment. (b) The Mandard tumor regression grade of nine patients who underwent prior treatment with chemoimmunotherapy.

Institutional Review Board approved all protocols of Jining No.1 People's Hospital, and all patients signed informed consent. The study was conducted following the ethical guidelines stated by the Jining No.1 People's Hospital. The patient characteristics are shown in Table 1.

TABLE 1 Baseline characteristics

Clinical features	Patients who received chemoimmunotherapy	Patients who did not receive chemoimmunotherapy
Gender		
male	7	7
female	2	2
age	60−77 (mean = 66.7)	55−74 (mean = 66.6)
T stage*		
T0	2	0
T1	4	2
T2	2	1
T3	1	6
T4	0	0
N stage		
N0	8	5
N1	1	2
N2	0	1
N3	0	1
Tumor diameter**	20−45 (mean = 33.4)	15−33 (mean = 28.3)

*T and N stages were all pathological stages after treatment in the treatment group.
**Tumor diameter refers to the maximum transverse diameter of the tumor, which was measured before treatment.

Immunohistochemistry (IHC) staining

All immunohistochemistry (IHC) staining was performed at the Boyuan Biological Laboratory. Whole section slides of FFPE tumor and paratumor were stained using a Dako instrument with PD-L1 (bs-1103R, Bioss, 1:200 dilution), IDO (13268-1-AP, Proteintech, 1:200 dilution), CD8(bs-10699R, Bioss, 1:200 dilution), TIM-3 (bs-10699R, Bioss, 1:200 dilution) and FOXP3+ (bs-10699R, Bioss, 1:200 dilution) antibodies. Whole section slides of FFPE lymph node were stained on a Dako instrument with PD-L1 (bs-1103R, Bioss, 1:200 dilution), IDO (13268-1-AP, Proteintech, 1:200 dilution) antibodies. All indices including PD-L1, IDO, TIM-3, CD8+, and FOXP3+ were stained Individually with corresponding antibody. The final staining results of the target protein were seen as a brown color, and the nucleus was blue-purple.

Expression density measurement

The integral topical density (IOD) and fluorescent area of CD8, PD-L1, IDO, TIM-3, and FOXP3+ were performed manually and averaged across three high power fields measuring 0.055 mm in diameter (10 × 22 mm ocular,

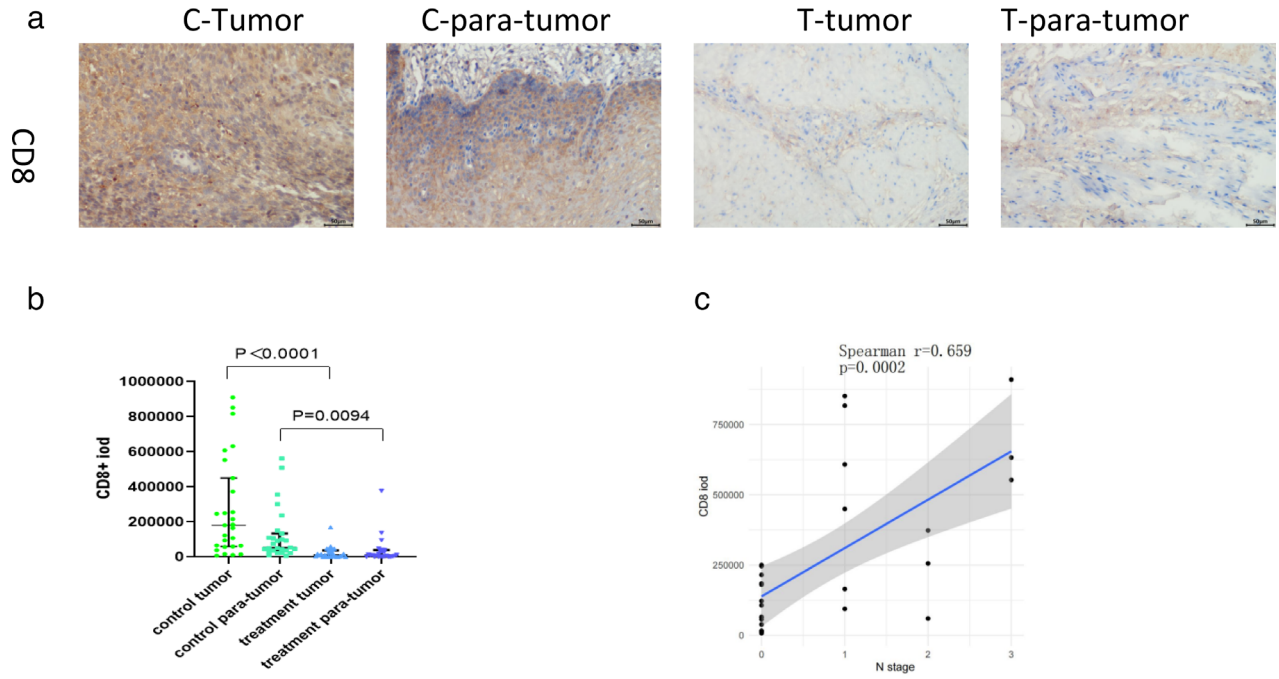

FIGURE 3 CD8 expression in different groups and tissues and the correlation of CD8 expression with N stage. (a) Representative images of CD8 (brown) and nucleus (blue) staining in one patient. CD8+ expression in control group tumor tissue (C-tumor), control group paratumor tissue (C-paratumor) and in the treatment group tumor tissue (T-tumor), and paratumor tissue (T-paratumor). (b) Decreased CD8 expression in patients treated preoperatively with chemoimmunotherapy (CI) as compared with patients treated preoperatively without CI in tumor ($p < 0.0001$) and paratumor tissues ($p = 0.0094$). Each dot represents one slide. (c) Correlation analysis between the cell densities for CD8 and N stage was performed using linear regression and correlation coefficient. CD8 IOD, CD8 integrated optical density; N stage, lymph node stage.

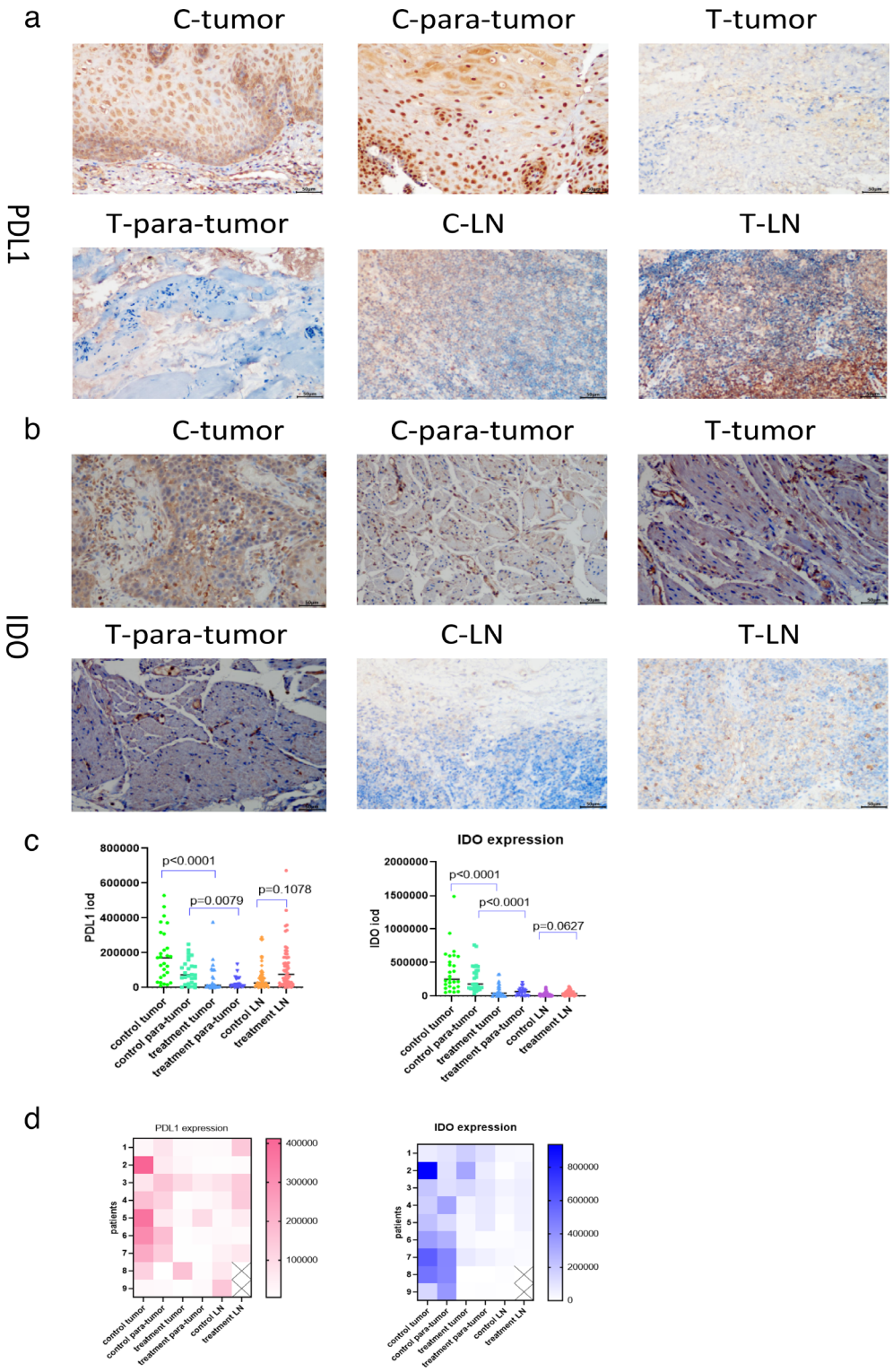

FIGURE 4 The differential expression of PD-L1 and IDO in different groups and tissues. (a) Representative images of PD-L1 (brown) and nucleus (blue) staining on tumor, paratumor and lymph node tissues in CI+ or CI- treatment patients. (b) Representative images of IDO (brown) and nucleus (blue) staining on tumor, paratumor and lymph node tissues in CI+ or CI- treatment patients. (c) Histograms illustrating the relationship between PD-L1 and IDO expression and prior treatment with chemoimmunotherapy (CI) in patients with ESCC, assayed across tumor, paratumor and lymph node (LN) tissues. CI pretreatment was associated with lower PD-L1, IDO expression in tumor ($p < 0.0001$, $p < 0.0001$) and paratumor tissues ($p = 0.0079$, $p < 0.0001$), not in LN tissues ($p = 0.1078$, $p = 0.0627$). (d) Heat map showing the PD-L1 and IDO protein expression in different patients.

400× objective). The initial field selected for each case was chosen based on the area of the highest expression density, after which the fields were selected based on the expression density. Whole section slides of FFPE tissues were measured by image analysis. The area and integral topical density (IOD) of fluorescence expression of each indicator were recorded.

Statistical analysis

The correlation of marker expression was evaluated by Spearman's correlation coefficient, and comparisons of medians were evaluated by Kruskal-Wallis test or Friedman's rank test. The sample size was calculated by PASS. Data were analyzed using the statistical software SPSS 18.0 (IBM), R version

3.5.3, and GraphPad Prism (GraphPad Software). The probability of clinical benefit from a PD-1 immune checkpoint inhibitor based on clinicopathological variables was examined by univariate and multivariate linear regression analyses. All statistical analyses were two-sided, and differences were considered statistically significant at $p < 0.05$.

RESULTS

CD8+ T cell expression is associated with tissue group and N stage

We investigated whether the CD8 integrated optical density (IOD) was associated with chemoimmunotherapy and clinical characteristics. Compared with patients without chemoimmunotherapy (CI), the expression of CD8+ T cells in both tumor ($p < 0.0001$) and paratumor ($p = 0.0094$) tissues was lower in patients with CI. However, there was no significant difference in CD8 expression between tumor and paratumor tissues ($p > 0.05$) in each respective group. Moreover, we investigated the relationship between CD8 expression and various clinicopathological characteristics, including the maximum transverse diameter of the tumor, gender, age, T, and N stages.

The multivariate analysis results revealed that only the N stage was significantly associated with CD8 expression in the concurrent control group (Figure 3).

PD-L1 and IDO protein expression in different groups and tissues

To investigate whether the expression of PD-L1 and IDO before and after anti-PD-1 antibody treatment was changed in patients with advanced esophageal squamous cell carcinoma, we analyzed the expression of PD-L1 and IDO by IHC and immunohistochemical optical density. PD-L1 and IDO expression in tumor ($p < 0.0001$, $p < 0.0001$) and paratumor ($p < 0.0079$, $p < 0.0001$) tissues was associated with the chances of experiencing CI treatment in both the treatment and concurrent control groups (Figure 4). However, lymph node tissues were similar between the two cohorts. Compared with patients without CI, PD-L1 and IDO expression was lower in patients with CI treatment. Within-group comparisons, no significant differences between tumor and paratumor tissues were found. In the concurrent control group, PD-L1 and IDO expression were clearly higher in tumor and paratumor tissues than in lymph node

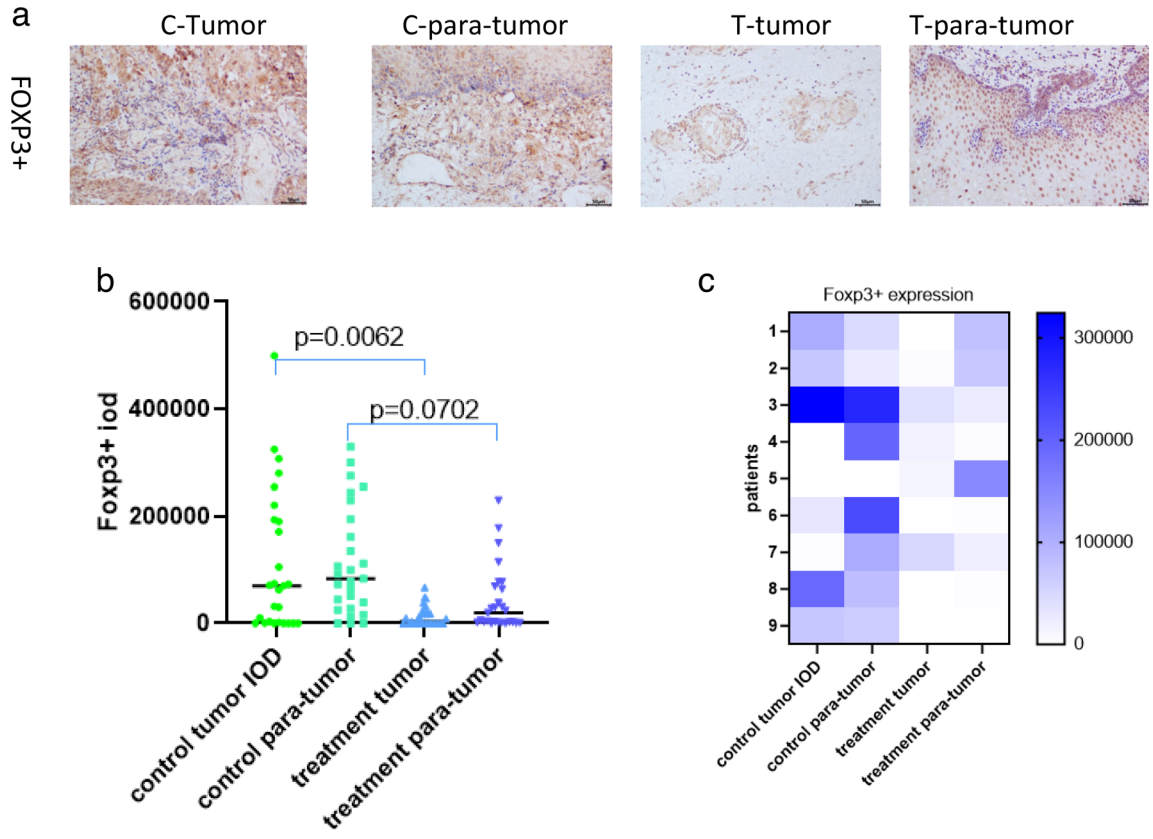

FIGURE 5 The differential expression of FOXP3+ in different groups. (a) Representative images of FOXP3+ staining (brown) in control group tumor tissue (C-tumor), control group paratumor tissue (C-paratumor) and in treatment group tumor tissue (T-tumor), paratumor tissue (T-paratumor). (b) Histograms illustrating the relationship between FOXP3+ expression and prior treatment with chemoimmunotherapy (CI) in patients with ESCC, assayed across tumor (T), and paratumor (PT) tissues. CI pretreatment (CI+) was associated with lower PD-L1 expression in tumor tissues ($p = 0.0062$), but no change in paratumor tissues ($p = 0.0702$). (c) FOXP3+ protein expression in different patients.

tissues. Nevertheless, in the treatment group, PD-L1 expression was significantly lower in tumor and paratumor tissues than in lymph node tissues, while IDO expression was not a significant difference in tumor and paratumor tissues than in lymph node tissues. After CI treatment, PD-L1 and IDO expression were significantly decreased in tumor and paratumor tissues, while it was unchanged in lymph node tissues.

FOXP3+ protein expression in different groups and tissues

To assess the different expression profiles of FOXP3+ between paratumor and tumor tissues, IHC experiments and immunohistochemical optical density were conducted on the total FOXP3+ protein obtained from samples. After a series of CI treatments for two or three cycles, significant decreases were observed in FOXP3+ expression in tumor tissues of the treatment group ($p = 0.0062$) compared to the concurrent control group. However, no significant variations were observed in paratumor tissues after CI treatment between the two groups ($p = 0.0702$). In addition, there were no differences between the two groups in tumor and paratumor tissues (Figure 5).

TIM-3 protein expression in different groups and tissues

To examine whether TIM-3 expression significantly differed in esophageal tumor and paratumor tissues, the effect of CI treatment on the expression level of TIM-3 was analyzed by IHC and immunohistochemical optical density. Compared with patients without CI treatment, significant decreases were observed in TIM-3 expression in paratumor tissues in patients with CI treatment ($p < 0.0001$). However, no significant variations were observed in tumor tissues after CI treatment between the two groups ($p = 0.7569$). Moreover, the expression of TIM-3 was not significantly different in tumor and paratumor tissues within the two groups (Figure 6).

Correlation analyses among markers in the concurrent control group

Correlation analyses of these markers, including PD-L1, IDO, TIM-3, FOXP3+, and CD8 T cells, were conducted in our study with Spearman's correlation test and rank-sum test.

In the control group, we noted that the PD-L1 expression level in tumor tissue has significant positive correlations with the expression levels of IDO ($r = 0.4371$ and

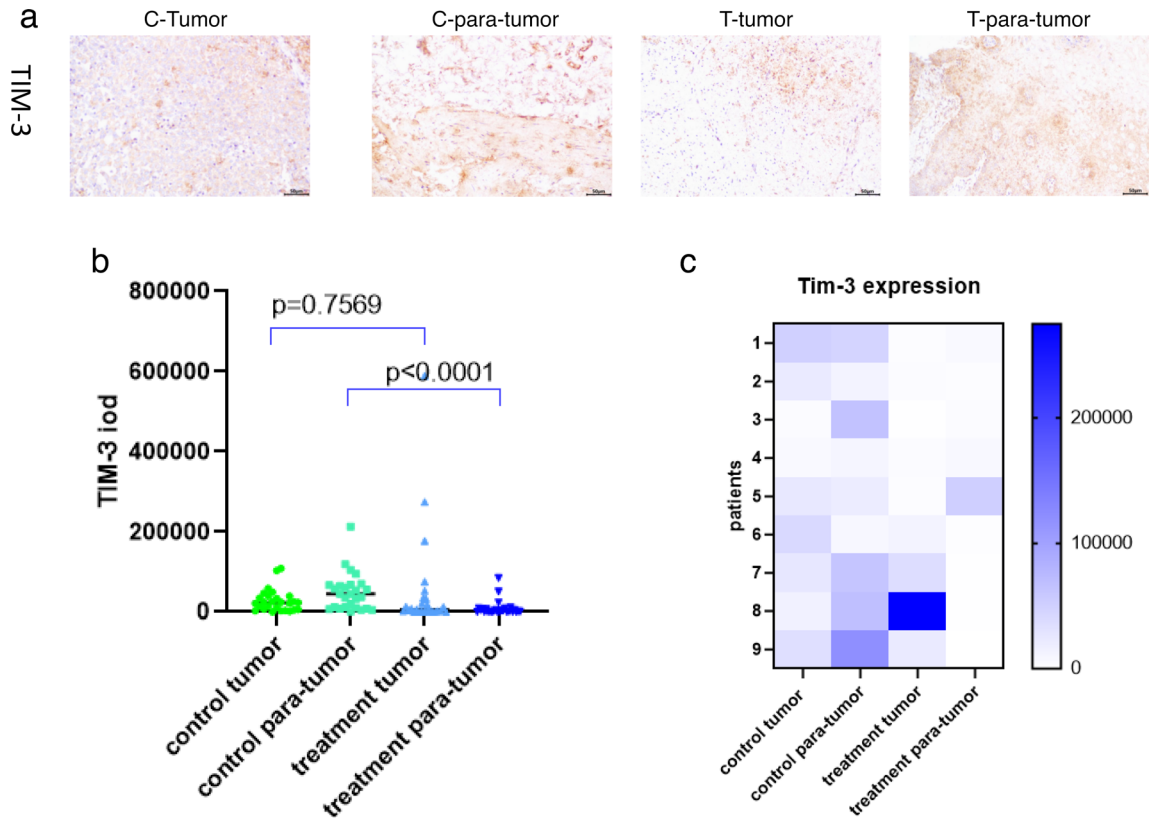

FIGURE 6 The differential expression of TIM-3 in different groups. (a) Representative images of TIM-3 staining (brown) in control group tumor tissue (C-tumor), control group paratumor tissue (C-paratumor) and in treatment group tumor tissue (T-tumor), paratumor tissue (T-para-tumor). (b) Histograms illustrating the relationship between tim-3 expression and prior treatment with chemoimmunotherapy (CI) in patients with esophageal squamous cell carcinoma (ESCC), assayed across tumor, paratumor tissues. CI pretreatment was associated with lower TIM-3 expression in paratumor tissues ($p < 0.0001$), but no change in tumor tissues ($p = 0.7569$). (c) TIM-3 protein expression in different patients.

$p = 0.0236$), and negative correlations with the FOXP3+ expression ($r = -0.4483$, $p = 0.019$) (Figure 7a). However, in paratumor tissues, correlations between PD-L1 and FOXP3+ expressions showed a high correlation of 0.5263 ($p = 0.0048$) (Figure 7b). No correlation was found between the other indicators ($p > 0.05$). Spearman's correlation coefficient revealed a significant reverse correlation between the expression levels of PD-L1 and FOXP3+ in tumor and paratumor tissues.

Correlation analyses among markers in treatment group

Our study indicated that by changing TME, CI treatment modifies these markers and the correlation of these markers. Spearman's correlation and rank-sum tests were conducted to provide further insight into the change.

In the tumor tissue of the treatment group, a strong positive correlation was seen between CD8+ T cells and IDO

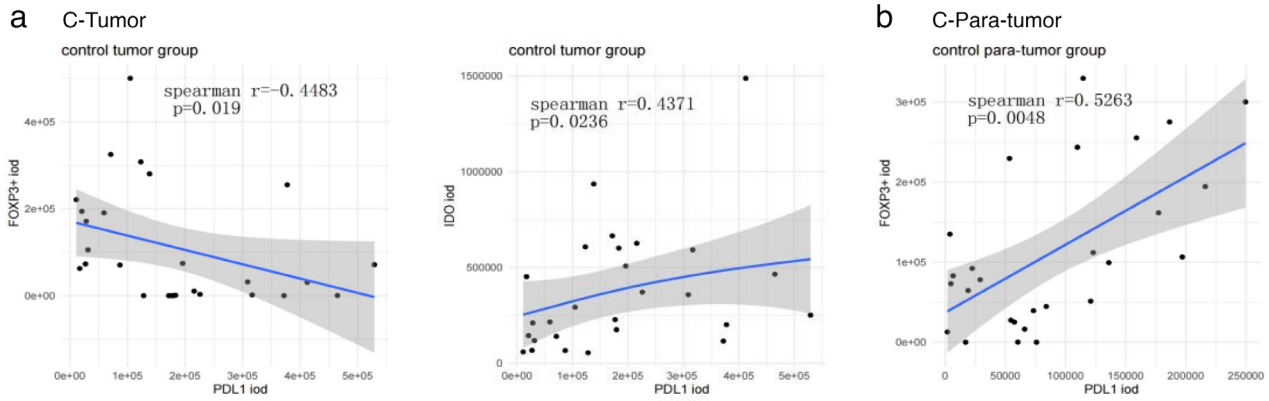

FIGURE 7 The correlation of PD-L1, FOXP3+, IDO, TIM-3, CD8 expression in nontreated esophageal squamous cell carcinoma (ESCC) patients. (a) Correlation between the integrated optical densities of PD-L1 and FOXP3+ (a, Spearman's R = −0.4483, $p = 0.019$), IDO (b, Spearman's R = −0.4371, $p = 0.0236$) in nontreated ESCC paratumor tissues of patients. (b) Correlation analysis between the integrated optical densities for PD-L1 and FOXP3+ was performed using linear regression and correlation coefficient (Spearman's R = 0.5263, $p = 0.0048$), in nontreated ESCC paratumor tissues of patients.

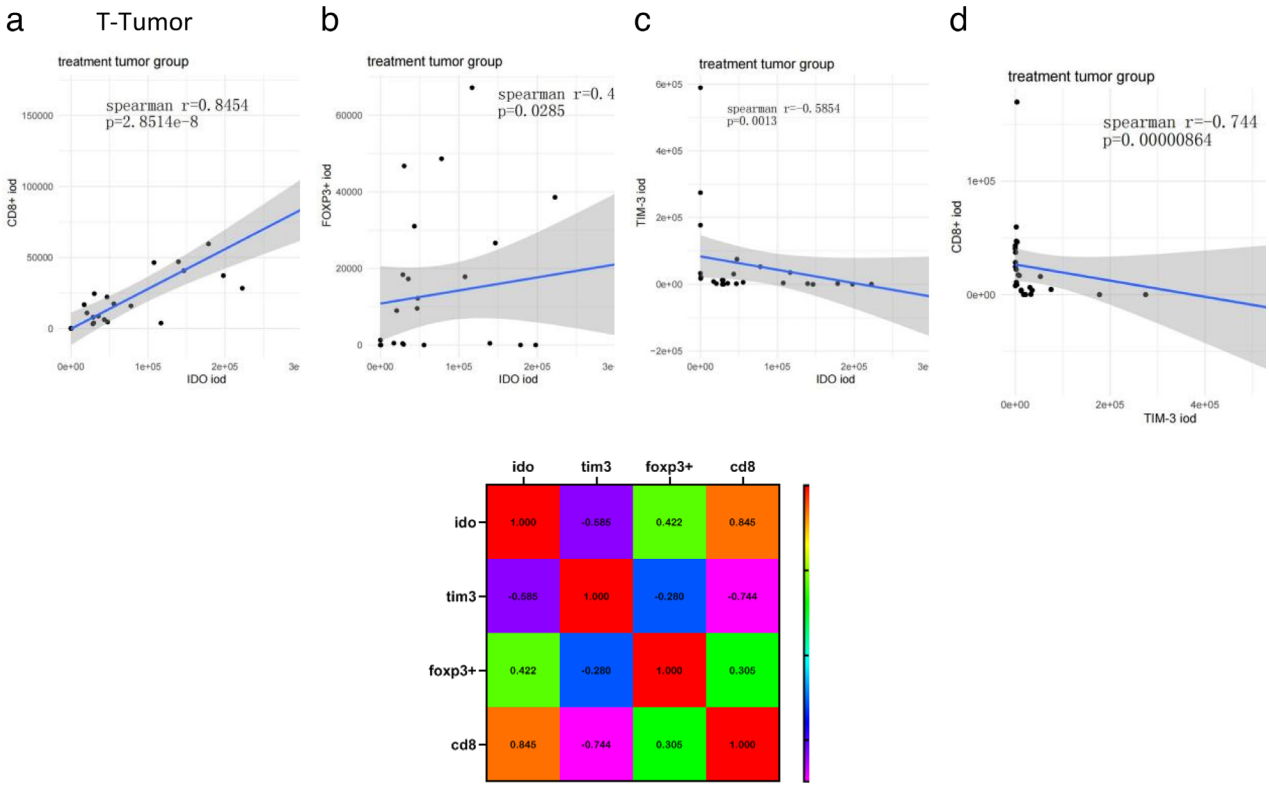

FIGURE 8 Correlation among CD8, IDO and TIM-3, FOXP3+ expression in treatment group tumor tissue. (a) A high positive correlation was observed between CD8 and IDO expression (Spearman's R = 0.8454, $p = 2.8514e-8$). (b) A moderate positive correlation was observed between IDO and FOXP3+ expression (Spearman's R = 0.4217, $p = 0.0285$). (c) A high negative correlation was observed between TIM-3 and IDO expression (Spearman's R = −0.5854, $p = 0.0013$). (d) A high negative correlation was observed between TIM-3 and CD8 (Spearman's R = −0.744, $p = 0.00000864$).

expression ($r = 0.8454$, $p = 2.8514e\text{-}8$), and a weak positive correlation was seen between FOXP3+ and IDO expression ($r = 0.4217$, $p = 0.0285$). Moreover, there was a modest inverse correlation between TIM-3 and CD8+ expression ($r = -0.5854$, $p = 0.0013$) and a marked negative correlation between TIM-3 and IDO expression ($r = -0.744$, $p = 0.00000864$). No obvious correlation among other markers was observed (Figure 8).

In the paratumor tissues of the treatment group, a strong positive correlation between CD8 T cells ($r = 0.5153$, $p = 0.0059$), FOXP3+ expression ($r = 0.4911$, $p = 0.0093$) and IDO expression and, independently, a weak positive correlation between TIM-3 expression level ($r = 0.4303$, $p = 0.0251$) and IDO expression. Moreover, the TIM-3 expression level had significant positive correlations with infiltrating levels of CD8+ T cells ($r = 0.5925$, $p = 0.0011$), FOXP3+ expression ($r = 0.653$, $p = 0.0002$). In our study, all biomarkers were demonstrated to have some degree of positive correlation with each other (Figure 9).

Compared with the control group, after PD-1 inhibitor and chemotherapy treatment, no correlation was found between PD-L1 and other markers in the treatment group, which indicated that by using CI treatment, PD-L1 expressions were changed.

DISCUSSION

Due to its heterogeneity, ESCC has inherent resistance to chemotherapy, and its effect is not ideal, with a low effective rate and easy drug resistance. There is a strong need to develop new therapeutic strategies for esophageal carcinoma. As a new method, immunotherapy has a great application prospect in esophageal cancer, especially in esophageal squamous cell carcinoma, and its combination with chemotherapy will produce synergistic reinforcement.[24–26] To further understand the mechanism of chemotherapy combined with immunotherapy in vivo, we evaluated the expression changes of multiple immune checkpoint molecules and cells in ESCC tumor TME, including CD8, PD-L1, TIM-3, IDO, and FOXP3+, compared to a control group. In attempting to provide better functional insight into the differences between the treatment and the control groups, we used IHC and immunohistochemical optical density to verify whether prior CI treatment was associated with broad differences in these immune checkpoint molecules and cell expression within the tumor microenvironment (TME) or diversity in adjacent nontumor regions.

Three new findings were obtained in our study: (1) The expression of markers such as CD8+ T cell infiltration, PD-L1, IDO, FOXP3+, and TIM-3, were affected by CI

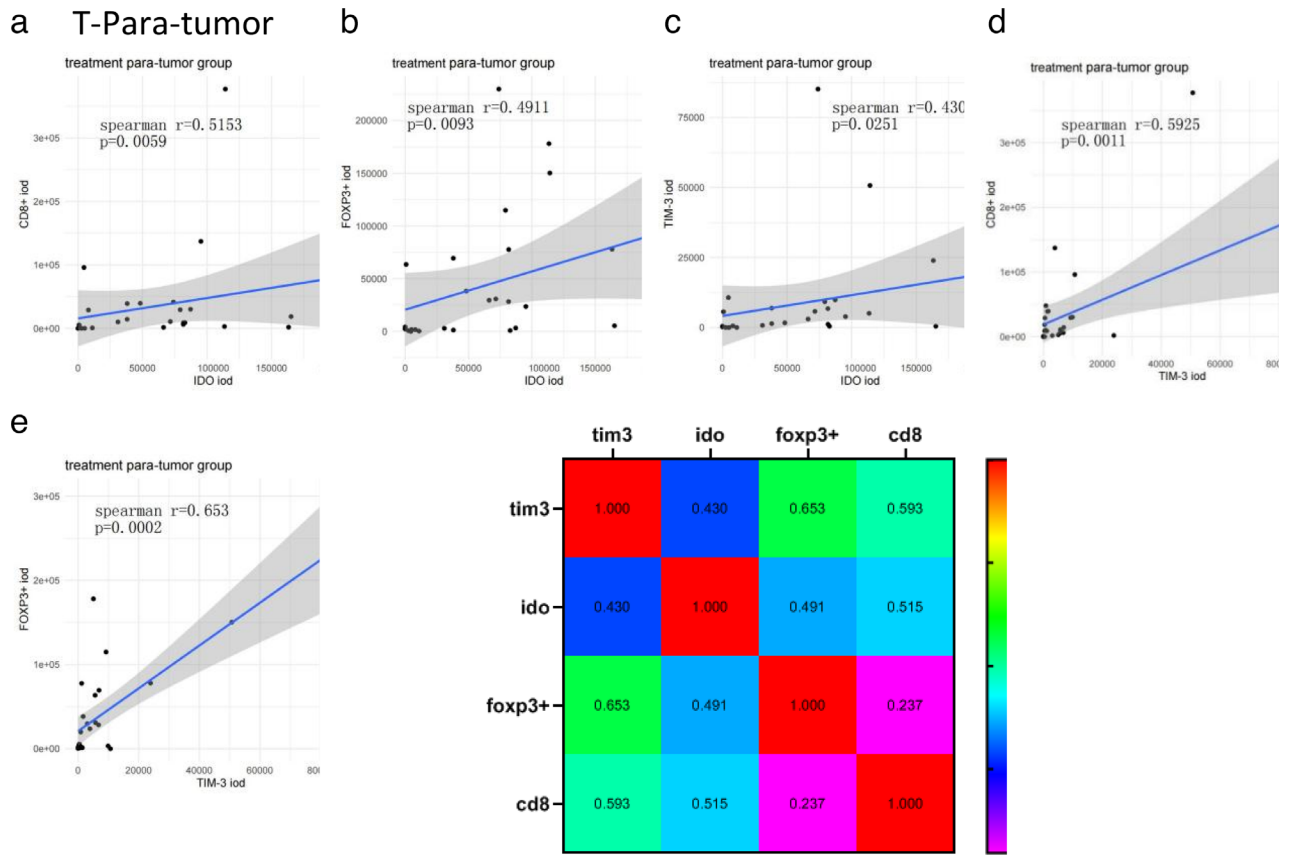

FIGURE 9 Correlation among CD8, IDO and TIM-3, FOXP3+ expression in treatment paratumor tissue. (a) A moderate positive correlation was observed between IDO and CD8 (Spearman's R = 0.5153, $p = 0.0059$). (b) A moderate positive correlation was observed between IDO and FOXP3+ expression (Spearman's R = 0.4217, $p = 0.0285$). (c) A moderate positive correlation was observed between IDO and TIM-3 expression (Spearman's R = 0.4303, $p = 0.0251$). (d) A high positive correlation was observed between TIM-3 and CD8 expression (Spearman's R = 0.5925, $p = 0.0011$). (e) A high positive correlation was observed between TIM-3 and FOXP3+ expression (Spearman's R = 0.635, $p = 0.0002$)

treatment. (2) The expressed levels of these markers varied significantly across individual tissues, and the difference was especially changed after CI treatment. (3) The results of our study indicated a correlation between these markers, which could also be affected by CI treatment.

The tumor microenvironment comprises immune cells, signaling molecules, and the extracellular matrix (ECM),[27] which modulates tumor growth and progression. The expression of these immune cells and signaling molecules can be influenced by chemotherapy or immunotherapy. However, the effects exerted by chemoimmunotherapy in the ESCC TME on these immune cells and signaling molecules are still unclear. Given the differences in the immune microenvironment in different tissues, the impact of CI treatment will also not be the same in different tissue and markers.[28] Therefore, this study aimed to provide new insight into the effects of CI treatment on isolated CD8+ T cell infiltration, PD-L1, IDO, FOXP3+, and TIM-3 expression as well as on their cross-talk in different tissues. Furthermore, through this, we could fully understand the impact of CI treatment on the immune microenvironment.

Before CI treatment, the PD-L1 and IDO expression was high in the tumor and paratumor tissues and low in the lymph nodes. Marked differences in PD-L1 expression were observed only between the lymph node and tumor tissues, while apparent differences in IDO expression were observed between tumor, paratumor tissues, and lymph nodes. After CI treatment, our data indicate that PD-L1 and IDO expression in tumor and paratumor tissues were significantly downregulated, and there was no differential expression in lymph node tissues.

Expression of CD8, FOXp3+, and TIM-3 had been observed in tumor and paratumor tissues, and no significant differences were seen in patients in the concurrent control group. The expression of FOXP3+ was apparently downregulated in the tumor after CI treatment and not in paratumor tissues, while the expression of TIM-3 was significantly downregulated in paratumor after CI treatment and not in tumor tissues. Moreover, the expression of CD8 was also markedly decreased in both tumor and paratumor tissues. Moreover, one thing that deserved special attention was that the enhancement of CD8 expression correlated with advanced N stages in the concurrent control group.

Previous studies have indicated that the expression of PD-L1, CD8+ TLS or IDO, TIM-3, and IFN-r was significantly upregulated in response to treatment with chemotherapy or immunotherapy.[10–12] Nevertheless, the findings from this investigation of CD8+, TLS, IDO, PD-L1, TIM-3, and FOXP3+ expression decreasing in tumor and/or paratumor tissues disagree with previous research. This may be due partly to the experimental combination therapy and different tumor types.

The correlation of these markers could be perturbed by CI treatment. Prior to CI treatment, in tumor tissues, PD-L1 expression was negatively associated with FOXP3+ while positively associated with IDO expression. In paratumor tissues, the expression of PD-L1 was only positively associated with FOXP3+ expression. After CI treatment, the expression of IDO was positively associated with CD8+, FOXP3+ expression, while TIM-3 expression was negatively associated with CD8+, IDO expression in tumor tissues. However, in paratumor tissues, IDO expression was positively associated with CD8, FOXP3+ expression, and TIM-3 expression was also positively associated with CD8, IDO, and FOXP3+ expression.

In our study, the correlation between PD-L1 expression in tumor or paratumor tissues and other markers disappeared after chemoimmunotherapy. Compared to patients without CI treatment, intermarker correlations excluding PD-L1 became apparent, indicating that these markers varied among therapies and tissues. Interestingly, our study found that the expression of PD-L1 and IDO in lymph node tissue did not correlate with the CI treatment, which indicated that different tissues were not equally sensitive to CI treatment.

The current study had several limitations. Specifically, this was a retrospective, case–control study with a small sample size of patients. Unfortunately, none of the patients in this study had pre-CI treatment tumor biopsies available for analysis. Therefore, we analyzed studies with contemporaneous control groups. While we attempted to minimize bias by including all patients who matched the protocol's eligibility criteria, selection bias remains possible.

In conclusion, despite these limitations, this is one of few studies to comprehensively evaluate the influence of CI treatment on the esophageal TME and LN in resected specimens. The expression of these cells or molecules in TME, including CD8+ TILS, IDO, PD-L1, TIM-3, and FOXP3+, was downregulated after CI treatment. Furthermore, there appeared to be a fundamental shift in the correlation of these markers. CD8 showed a strong positive correlation with IDO and a negative correlation with TIM-3 in tumor tissues after CI treatment. These biomarkers were highly positively correlated in nontumor tissues after CI treatment. Moreover, N staging was associated with high expression of CD8 in advanced esophageal squamous cell carcinoma in the concurrent control group.

FUNDING INFORMATION

This work was Sponsored by the Doctoral Fund of Jining No.1 People's Hospital.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Shifa Zhang https://orcid.org/0000-0002-7361-9336

REFERENCES

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108. https://doi.org/10.3322/caac.21262
2. Hongo M, Nagasaki Y, Shoji T. Epidemiology of esophageal cancer: orient to occident. Effects of chronology, geography and ethnicity. J Gastroenterol Hepatol. 2009;24(5):729–35. https://doi.org/10.1111/j.1440-1746.2009.05824.x
3. Eyck BM, van Lanschot JJB, Hulshof MCCM, Steyerberg EW, van der Gaast A, et al. CROSS study group. Ten-year outcome of neoadjuvant

chemoradiotherapy plus surgery for esophageal cancer: the randomized controlled CROSS trial. J Clin Oncol. 2021;39(18):1995–2004. https://doi.org/10.1200/JCO.20.03614
4. Sjoquist KM, Burmeister BH, Smithers BM, Zalcberg JR, Simes RJ, Barbour A, et al. Australasian gastro-intestinal trials group. Survival after neoadjuvant chemotherapy or chemoradiotherapy for resectable oesophageal carcinoma: an updated meta-analysis. Lancet Oncol. 2011;12(7):681–92. https://doi.org/10.1016/S1470-2045(11)70142-5
5. Kamarajah SK, Phillips AW, Ferri L, Hofstetter WL, Markar SR. Neoadjuvant chemoradiotherapy or chemotherapy alone for oesophageal cancer: population-based cohort study. Br J Surg. 2021;108(4):403–11. https://doi.org/10.1093/bjs/znaa121 PMID: 33755097.
6. Luo H, Lu J, Bai Y, Mao T, Wang J, Xu RH, et al. Effect of Camrelizumab vs placebo added to chemotherapy on survival and progression-free survival in patients with advanced or metastatic esophageal squamous cell carcinoma: the ESCORT-1st randomized clinical trial. JAMA. 2021;326(10):916–25. https://doi.org/10.1001/jama.2021.12836 PMID: 34519801; PMCID: PMC8441593.
7. Kato K, Sun J, Shah MA, Enzinger PC, Adenis A, Doi T, et al. Pembrolizumab plus chemotherapy versus chemotherapy as first-line therapy in patients with advanced esophageal cancer: the phase 3 KEYNOTE-590 study. Ann Oncol. 2020;31(Suppl_4):S1142–215. https://doi.org/10.1016/j.annonc.2020.08.2298
8. Kato K, Cho BC, Takahashi M, Okada M, Lin CY, Chin K, et al. Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy (ATTRACTION-3): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol. 2019;20(11):1506–17. https://doi.org/10.1016/S1470-2045(19)30626-6
9. Katsuya Y, Horinouchi H, Asao T, Ohe Y, et al. Expression of programmed death 1 (PD-1) and its ligand (PD-L1) in thymic epithelial tumors: impact on treatment efficacy and alteration in expression after chemotherapy. Lung Cancer. 2016;99:4–10. https://doi.org/10.1016/j.lungcan.2016.05.007 Epub 2016 May 12. PMID: 27565906.
10. Fukuoka E, Yamashita K, Tanaka T, Kakeji Y, et al. Neoadjuvant chemotherapy increases PD-L1 expression and CD8+ tumor-infiltrating lymphocytes in esophageal squamous cell carcinoma. Anticancer Res. 2019;39(8):4539–48. https://doi.org/10.21873/anticanres.13631 PMID: 31366557.
11. Shayan G, Srivastava R, Li J, Schmitt N, Kane LP, Ferris RL. Adaptive resistance to anti-PD1 therapy by Tim-3 upregulation is mediated by the PI3K-Akt pathway in head and neck cancer. Onco Targets Ther. 2016;6(1):e1261779. https://doi.org/10.1080/2162402X.2016.1261779 PMID: 28197389; PMCID: PMC5283618.
12. Ji RR, Chasalow SD, Wang L, Shahabi V, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother. 2012;61(7):1019–31. https://doi.org/10.1007/s00262-011-1172-6 Epub 2011 Dec 7. PMID: 22146893.
13. Taube JM, Galon J, Sholl LM, Cimino-Mathews A, et al. Implications of the tumor immune microenvironment for staging and therapeutics. Mod Pathol. 2018;31(2):214–34. https://doi.org/10.1038/modpathol.2017.156 Epub 2017 Dec 1. PMID: 29192647; PMCID: PMC6132263.
14. Powles T, Eder JP, Fine GD, Vogelzang NJ, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature. 2014;515(7528):558–62. https://doi.org/10.1038/nature13904 PMID: 25428503.
15. Tumeh PC, Harview CL, Yearley JH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568–71. https://doi.org/10.1038/nature13954 PMID: 25428505; PMCID: PMC4246418.
16. Maleki Vareki S, Chen D, Di Cresce C, Koropatnick J, et al. IDO downregulation induces sensitivity to pemetrexed, gemcitabine, FK866, and methoxyamine in human cancer cells. PLoS One. 2015;10(11):e0143435. https://doi.org/10.1371/journal.pone.0143435 PMID: 26579709; PMCID: PMC4651508.
17. Botticelli A, Cerbelli B, Lionetto L, Marchetti P, et al. Can IDO activity predict primary resistance to anti-PD-1 treatment in NSCLC. J Transl Med. 2018;16(1):219. https://doi.org/10.1186/s12967-018-1595-3 PMID: 30081936; PMCID: PMC6080500.
18. Sehrawat S, Reddy PB, Rajasagi N, Suryawanshi A, Hirashima M, Rouse BT. Galectin-9/TIM-3 interaction regulates virus-specific primary and memory CD8 T cell response. PLoS Pathog. 2010;6(5):e1000882. https://doi.org/10.1371/journal.ppat.1000882 PMID: 20463811; PMCID: PMC2865527.
19. Zhu C, Anderson AC, Schubart A, Kuchroo VK. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol. 2005;6(12):1245–52. https://doi.org/10.1038/ni1271 Epub 2005 Nov 13. PMID: 16286920.
20. Freeman GJ, Casasnovas JM, Umetsu DT, DeKruyff RH. TIM genes: a family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity. Immunol Rev. 2010;235(1):172–89. https://doi.org/10.1111/j.0105-2896.2010.00903.x PMID: 20536563; PMCID: PMC2914464.
21. Huang YH, Zhu C, Kondo Y, Blumberg RS, et al. Corrigendum: CEACAM1 regulates TIM-3-mediated tolerance and exhaustion. Nature. 2016;536(7616):359. https://doi.org/10.1038/nature17421 Epub 2016 Mar 16. Erratum for: Nature. 2015 Jan 15;517(7534):386–90. PMID: 26982724; PMCID: PMC5110397.
22. Tang D, Lotze MT. Tumor immunity times out: TIM-3 and HMGB1. Nat Immunol. 2012;13(9):808–10. https://doi.org/10.1038/ni.2396 PMID: 22910384; PMCID: PMC3672065.
23. Yang R, Sun L, Li CF, Hung MC, et al. Galectin-9 interacts with PD-1 and TIM-3 to regulate T cell death and is a target for cancer immunotherapy. Nat Commun. 2021;12(1):832. https://doi.org/10.1038/s41467-021-21099-2 PMID: 33547304; PMCID: PMC7864927.
24. Salas-Benito D, Perez-Gracia JL, Ponz-Sarvise M, Rodriguez-Ruiz ME, Martinez-Forero I, Castanon E, et al. Paradigms on immunotherapy combinations with chemotherapy. Cancer Discov. 2021;11(6):1353–67. https://doi.org/10.1158/2159-8290 CD-20-1312.
25. Yamamoto S, KatoK DH, et al. Feasibility study of nivolumab as neoadjuvant chemotherapy for locally esophageal carcinoma: FRONTiER (JCOG1804E)[J]. Future Oncol. 2020;16(19):1351–7. https://doi.org/10.2217/fon-2020-0189
26. Sun JM, Shen L, Shah MA, Bhagia P, Kato K. Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): a randomised, placebo-controlled, phase 3 study. Lancet. 2021;398(10302):759–71. https://doi.org/10.1016/S0140-6736(21)01234-4 Erratum in: Lancet. 2021 Nov 20;398(10314):1874. PMID: 34454674.
27. Spill F, Reynolds DS, Kamm RD, Zaman MH. Impact of the physical microenvironment on tumor progression and metastasis. Curr Opin Biotechnol. 2016;40:41–8. https://doi.org/10.1016/j.copbio.2016.02.007 Epub 2016 Mar 2. PMID: 26938687; PMCID: PMC4975620.
28. Jiao S, Subudhi SK, Aparicio A, Ge Z, Guan B, Miura Y, et al. Differences in tumor microenvironment dictate T helper lineage polarization and response to immune checkpoint therapy. Cell. 2019;179(5):1177–90. https://doi.org/10.1016/j.cell.2019.10.029 PMID: 31730856.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Zhang S, Cai H, Huang J, Wang G. Impact of the combination of sintilimab and chemotherapy on the tumor and paratumor PD-L1, IDO, TIM-3, FOXP3+ and CD8 expressions in patients with advanced esophageal squamous cell carcinoma. Thorac Cancer. 2022;13(23):3284–94. https://doi.org/10.1111/1759-7714.14683