IVYSPRING INTERNATIONAL PUBLISHER

Journal of Cancer

2018; 9(12): 2224-2231. doi: 10.7150/jca.24493

Research Paper

PD-L1 Expression On tumor Cells Was Associated With Unfavorable Prognosis In Esophageal Squamous Cell Carcinoma

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Received: 2017.12.21; Accepted: 2018.03.26; Published: 2018.06.05

Abstract

Background: Evidence about the association between programmed cell death ligand 1 (PD-L1) expression and prognosis of esophageal squamous cell carcinoma (ESCC) were limited and controversial. Thus, the present study aims to investigate the prognostic value of tumor immune microenvironment (TIM) based on PD-L1 expression and CD8+ T cell infiltration in ESCC tissues.

Methods: From September 2008 to March 2010, a total of 146 ESCC patients received radical esophagectomy were retrospectively analyzed in our present study. PD-L1 expression and CD8+ T cell infiltration were evaluated through immunohistochemistry. The clinicopathological characteristics and survival were analyzed.

Results: There were 111 male and 35 female. The median age was 59.1 years (37-78 years). The positive rate of PD-L1 expression was 61.7%. The rate of high CD8+ T cell infiltration was 33%. No significant differences were found between clinicopathological features and PD-L1 expression or CD8+ T cell infiltration. PD-L1 expression was significantly associated with poor overall survival (P=0.010). However, CD8+ T cell infiltration was not a prognostic risk factor. Type of TIM was significantly associated with the prognosis of ESCC patients (P=0.021).

Conclusions: PD-L1 expression was an independent risk factor for the prognosis of ESCC patients. Immunotherapy may achieve promising outcomes in ESCC patients with type I TIM.

Key words: esophageal squamous cell carcinoma, programmed cell death ligand 1, tumor immune microenvironment, prognosis

Introduction

ESCC is the sixth most common cancer and is ranked fourth in the cancer related mortality in China. Commonly, the incidence of ESCC is highest in central China, and the clinicopathological features are different from the West [1,2]. Although multidisciplinary therapies have been applied to the treatment of ESCC, the prognosis of patients is still not promising [2].

PD-L1 belongs to the B7 super-family, which is expressed on activated T cells, B cells, dendritic cells, macrophages, and also on tumor cells [3,4]. PD-1 is expressed on the surface of immune cells. The binding of PD-L1/PD-1 could enable tumor cells to avoid antitumor immunity [5]. Thus, the immunotherapy, targeting the PD-L1/PD-1 immune checkpoint, has been an emerging field. Several clinical trials have

shown promising antitumor activity of PD-L1/PD-1 blockade in several malignancies [6-11]. However, not all patients obtained favorable response and durable efficacy [12,13]. This may attribute to the different type of TIM in tumor tissues. Based on the expression of PD-L1 on tumor cells and CD8+ T cell infiltration in tumor tissues, TIM was classified into four types: type I (PD-L1+/CD8 High, adaptive immune resistance), type II (PD-L1-/CD8 Low, immune ignorance type), type III (PD-L1+/CD8 Low, intrinsic induction of PD-L1 in the absence of TILs), and type IV (PD-L1-/CD8 High, components other than PD-L1 suppressing the action of TILs) [14]. It was reported that type of TIM was a potentially powerful biomarker for the prognosis of solid cancers [15], and type I TIM have been demonstrated to be a potential subgroup for anti-PD-1 or anti-PD-L1 immunotherapy [7,16,17].

Recently, a series of studies have investigated the prognostic value of expression of PD-L1 on tumor cells in ESCC patients. However, the findings were controversy. Most studies reported that PD-L1 expression was associated with poor prognosis of ESCC patients [18-20]. However, a few investigations reported the opposite results [21,22]. Up to date, the prognostic value of TIM in ESCC patients has not been investigated yet. Thus, the present study co-assessed PD-L1 expression and CD8+ T cell infiltration in ESCC, and investigated the prognostic value of PD-L1 expression and type of TIM in ESCC.

Materials and methods

Patients

This study was performed in the Xijing Hospital of Digestive Diseases affiliated to the Fourth Military Medical University. From September 2008 to March 2010, a total of 146 ESCC patients were enrolled in our present study. The inclusion criteria were listed as follows: 1. treated with radical esophagectomy, 2. with follow up data. The exclusion criteria were: 1. with neoadjuvant chemotherapy, 2. with distant metastasis, 3. with other malignancies. The clinicopathological data including age, gender, tumor location, tumor size, differentiation status, tumor depth, lymph node metastasis and TNM stage were recorded. The tumors were staged according to the seventh edition of the American Joint Committee on Cancer TNM classification. The patients were followed up until November 2016 by enhanced CT every 3 months. This study was approved by the Ethics Committee of Xijing Hospital, and written informed consent was obtained from all patients before surgery.

Immunohistochemistry

The tissues were fixed with 4% formaldehyde, embedded in paraffin and sectioned serially at 4 μm thickness. Briefly, the sections were deparaffinized in xylene, dehydrated with graded ethanol, pretreated in citrate buffer with 2 minutes in a pressure cooker for antigen retrieval, and then blocked with 3% H_2O_2 for 10 mins, goat serum for 10 mins. After blocking, the sections were incubated with rabbit anti-PD-L1 monoclonal antibody (1:200, ab13684S, Cell Signaling Technology, USA) or anti-CD8+ monoclonal antibody (1:50, clone: C8/144B, ZSDB, China) at 4°C overnight, and then incubated with HRP-Polymer anti-rabbit IHC Kit (Fuzhou Maixin Biotechnology, China) at room temperature for 30 minutes according to the manufacturer's instructions.

Evaluation of immunostaining

All tissue slides were evaluated by 3 independent pathologists. The immunoreactivity scoring system (IRS) was calculated based on the intensity category and percentage category. The intensity category of immunostaining was graded as follows: 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The percentage category was graded as follows: 0 (negative), 1 (1%-30%), 2 (31% to 60%) and 3 (61%-100%). The IRS was calculated by multiplication of both categories [23]. Then, the expression of PD-L1 was classified as negative ($0 \leq IRS \leq 2$) and positive ($3 \leq IRS \leq 9$) based on IRS. In order to ensure the quality control during IHC evaluation, positive and negative control was used during IHC to exclude nonspecific staining[24-27]. Tissue slides were evaluated by 3 independent pathologists who were blinded to clinical outcomes. When the results were inconsistent, the results were discussed by the 3 independent pathologists to make a final evaluation. The numbers of CD8+ T cell were counted in 6-10 high power magnification (40x) field with the most abundant distribution of CD8+ cells within tumor. The average numbers of CD8+ T cell per high power magnification field (HPF) were recorded. The CD8 high and CD8 low groups were defined using the 66th percentile of the average as the cut-off value. Based on density of CD8+ T cell and expression of PD-L1, type of TIM was classified into four types: type I (PD-L1+/CD8 high), type II (PD-L1-/CD8 low), type III (PD-L1+/CD8 low) and type IV (PD-L1-/CD8 high).

Statistical analysis

Data were processed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Discrete variables were analyzed using Chi-square test or Fisher's exact test. Significant risk factors for the prognosis of ESCC patients identified by univariate