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Programmed death ligand 1 expression and CD8$^+$ tumor-infiltrating lymphocyte density differences between paired primary and brain metastatic lesions in non-small cell lung cancer

Jie Zhou [a, 1], Zhihua Gong [a, 1], Qingzhu Jia [a], Yan Wu [b], Zhen-Zhou Yang [c, **], Bo Zhu [a, *]

[a] Institute of Cancer, Xinqiao Hospital, Third Military Medical University, Chongqing, 400037, China
[b] Institute of Cancer Center, Research Institute of Surgery, Daping Hospital, Third Military Medical University, 400042, Chongqing, China
[c] Department of Oncology, The Second Affiliated Hospital, Chongqing Medical University, Chongqing, 400010, China

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ABSTRACT

Immunotherapy targeting the programmed cell death-1/programmed death ligand 1(PD-L1) pathway has shown promising antitumor activity in brain metastases (BMs) of non-small cell lung cancer (NSCLC) patients with an acceptable safety profile; however, the response rates often differ between primary lesions and intracranial lesions. Studies are necessary to identify detailed characterizations of the response biomarkers. In this study, we aimed to compare the differences of PD-L1 expression and CD8$^+$ tumor-infiltrating lymphocyte (TIL) density, two major response biomarkers of PD-1/PD-L1 blockade, between paired primary and brain metastatic lesions in advanced NSCLC. We observed that among primary lesions or BMs, only a small number of patients harbored common PD-L1 expression on both tumor cells and tumor-infiltrating immune cells. Additionally, we found that the numbers of CD8$^+$ TILs were significantly fewer in BMs than in primary lung cancers. Low stromal CD8$^+$ TIL numbers in BMs were associated with significantly shorter overall survival compared to high stromal CD8$^+$ TIL counts. Notably, we demonstrated a discrepancy in PD-L1 expression and CD8$^+$ TIL density between primary lung cancers and their corresponding BMs. Such heterogeneities are significantly associated with the time at which BMs occurred. Our study emphasizes the spatial and temporal heterogeneity of biomarkers for anti-PD-1/PD-L1 therapy, which should be concerned in clinical practice.

1. Introduction

Non-small cell lung cancer (NSCLC) accounts for 85–90% of lung cancers, which are the leading cause of cancer-related death globally [1]. One of the most frequent and serious complications of this ubiquitous disease is metastasis to the brain [2,3]. Therapy options for patients with NSCLC and brain metastases (BMs) remain limited and unsatisfactory, and the prognosis is very poor even after positive treatment [4,5]. The new development of antibodies that target programmed cell death-1 (PD-1) or programmed death ligand 1 (PD-L1) represents a vital improvement in metastatic NSCLC therapy, with PD-1 inhibitors demonstrating impressive antitumor activity against BMs in NSCLC patients. Nevertheless, the response rates of primary lung cancer and corresponding metastatic brain lesions are not exactly the same [6,7]. Understanding the potential reasons for this discordance can provide valuable clues as to improve patient selection and treatment outcomes.

PD-L1 (also known as B7-H1), an immune-inhibitory molecule, is known to be expressed on tumor cells (TCs) and tumor-infiltrating immune cells (ICs) that suppresses antitumoral T-cell function through binding to PD-1 and B7.1 receptors on activated T and B cells [8]. Based on the results from clinical trials, PD-L1 expression was identified to be a predictive biomarker of response to PD-1/PD-L1 blockade therapy [9–12]. Interestingly, growing evidence suggests that PD-L1 is a dynamic biomarker subject to changes due to the tumor microenvironment [13,14]. In line with this, a recent report showed that PD-L1 expression can be heterogenic depending on the metastatic sites and histological

* Corresponding author. No.83, Xinqiao Main Street, Shaping District, Chongqing, China.
** Corresponding author. No. 76, Linjiang Road, Yuzhong District, Chongqing, China.
E-mail addresses: yangzhenzhou@sohu.com (Z.-Z. Yang), b.davis.zhu@gmail.com (B. Zhu).
[1] These authors contributed equally to this work.

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transformation [15]. In this regard, a comprehensive assessment of PD-L1 expression is important to determine optimal therapeutic strategies. However, studies regarding the inter-tissue heterogeneity of PD-L1 expression in NSCLC BM patients is very limited.

PD-L1 expression in the tumor microenvironment is often associated with tumor-infiltrating lymphocytes (TILs), of which $CD8^+$ TILs may be the most potent. $CD8^+$ TILs can induce or maintain PD-L1 expression by secreting interferon (IFN)-γ [16]; on the other hand, PD-L1 expression also contributes to apoptosis of $CD8^+$ TILs [17]. It is noteworthy that besides PD-L1, pre-existing $CD8^+$ T cells within tumor lesions are also a potential therapeutic response marker of PD-1 pathway inhibitors [13,18]. Moreover, $CD8^+$ TIL density has been reported as a significant prognostic factor alone or in combination with other markers in a spectrum of different tumor types including NSCLC [19–21]. Given the dynamics of PD-L1 expression, we wonder if the $CD8^+$ TIL accumulation in tumors is also dynamic. To date, for NSCLC patients with BMs, whether the $CD8^+$ TIL density between primary tumors and their corresponding BMs is consistent remains less clear.

To address these issues, here, we retrospectively analyzed the prevalence and concordance of the PD-L1 expression between primary lesions and BMs in advanced NSCLC patients. We also compared the $CD8^+$ TIL counts within the cancer parenchyma and stroma between paired tumors and evaluated its prognostic value.

2. Materials and methods

2.1. Patients

The study included 25 patients with NSCLC and BMs from three academic medical centers of the Third Military Medical University (Chongqing, China), and was conducted between January 2006 and September 2014. Among the patients, 2 were diagnosed with NSCLC based on the pathological examination of biopsy specimens and underwent a surgical resection of the brain metastatic lesions (alone) to alleviate the symptoms. The remaining 23 patients underwent surgical resection of both the primary lung cancer and the BMs. Common clinical characteristics such as age at diagnosis, gender and smoking history (nonsmokers were defined as those who smoked less than 100 cigarettes in their lifetime) were retrospectively collected and are summarized in Table 1. According to previous reports [22], patients who were diagnosed with BM by radiological examination within 1 month of their initial pathological diagnosis of NSCLC were determined as NSCLC patients with synchronous BM. The rest of the patients were considered as having metachronous BM. Patients with a history of multiple malignancies were excluded to reduce the possibility of including cancers other than NSCLC.

This study was approved by the Ethics Committee of Xinqiao Hospital, Third Military Medical University (Chongqing, China). Written informed consent was obtained from each patient or their family member.

2.2. Immunohistochemical staining for PD-L1 and CD8

The formalin-fixed, paraffin-embedded specimens of the primary tumors and the BMs were subjected to an immunohistochemically analysis. PD-L1 expression was measured by the Shuwen Biotech Co. LTD (Zhejiang, China) with an investigational version of the Human PD-L1 Immunohistochemistry Kit using the 6E8 antibody (Shuwen Biotech Co., Ltd., Zhejiang Province, China) (In line with the American Society of Pathology CAP and ISO15189 quality management standards). PD-L1 staining with this antibody has been used in clinical trial [23] and well validated before starting this research (data not shown). CD8 staining was performed according to a previously published protocol [24]. CD8 monoclonal antibodies (MAB-0021, Maixin, Fujian, China, diluted 1:100) were used as primary antibodies, and a MaxVision HRP-Polymer anti-Mouse/Rabbit IHC Kit (KIT-5930; Maixin) was applied to visualize the antigen. PD-L1-positivity ($PD\text{-}L1^+$) was determined by a cut-off value of 5%, among $PD\text{-}L1^+$ cases, we also defined PD-L1 strong positivity ($PD\text{-}L1^{high}$) on TC as PD-L1 ≥ 50%, and defined $PD\text{-}L1^{high}$ expression on IC as PD-L1 ≥ 10%, according to previous clinical trials [9]. The numbers of $CD8^+$ cells were quantified (5 fields within the tumor parenchyma and 3 fields within the stroma) using high-power fields (400 ×). $CD8^+$ TIL accumulations were binarized using the mean value of $CD8^+$ TIL infiltration as a cut-off for low and high categories. For each sample, the expression of CD8 and PD-L1 were determined by two well-experienced pathologists (X.D. and J.C.) independently, without any prior information on the patients.

2.3. Statistical analysis

All statistical analyses were performed using GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA) and SPSS 20.0 software. Frequencies and descriptive statistics of demographic and clinical variables were obtained. A Fisher's exact test was used for categorical variables. For pairwise comparisons, a paired Student's *t*-test or Wilcoxon matched-pairs signed-rank tests were used. The Kaplan-Meier method was used for survival analyses, and the significance of differences between groups was evaluated by the log-rank test. A P-value < 0.05 was considered statistically significant.

3. Results

3.1. Patient demographics and clinicopathologic characteristics

The characteristics of 25 NSCLC BM patients were listed in Table 1. The median age of all patients was 57 years (range: 26–78 years). These patients tended to be male (72%), non-smokers (52%), and the Karnofsky Performance Status (KPS) score of most patients (80%) was not less than 80. All patients harbored brain metastatic lesion. Eleven (44%) of these patients were diagnosed with synchronous BM, and 14 patients (56%) were diagnosed with metachronous BM. Adenocarcinoma (80%) was the major histological type. Three patients (12%) were identified as harboring an *EGFR* mutation in the primary tumors and 6 patients (24%) harbored an *EGFR* mutation in the BMs.

3.2. Discordant PD-L1 expression between primary lung cancers and their corresponding BMs

PD-L1 expression of tumors from NSCLC BM patients were detected on both TCs and ICs by immunohistochemistry staining (Fig. 1A). As for primary lung cancer specimens, only 16% of patients demonstrated $PD\text{-}L1^+$ expression on both TCs and ICs (Fig. 1B), indicating that patients with $PD\text{-}1^+$ on TCs and patients with $PD\text{-}1^+$ on ICs are different subpopulations of metastatic NSCLC. At present, the prevalence of intracranial PD-L1 expression in NSCLC BM patients remains unclear. We found that among brain metastatic lesions, in addition to 32% of patients with $PD\text{-}L1^+$ expression on both TCs and ICs, there are different populations of patients with $PD\text{-}L1^+$ on TCs only or on ICs only (e.g., 8% of patients expressed PD-L1 on ICs only) (Fig. 1B). Further analysis of the $PD\text{-}L1^{high}$ expression pattern showed that only 4% of patients shared common $PD\text{-}L1^{high}$ expression on both TCs and ICs among primary lesions and 16% of patients were simultaneously detected as having a strong positive PD-L1 expression on TCs and ICs among brain metastatic lesions (Fig. 1C).