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Concordance of immune checkpoints within tumor immune contexture and their prognostic significance in gastric cancer

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ABSTRACT

Checkpoint blockade therapy has emerged as a novel approach for cancer immunotherapy in several malignancies. However, patient prognosis and disease progression relevant to immune checkpoints in gastric tumor microenvironment are not defined. This study aims to investigate the expression and prognostic significance of immune checkpoints within gastric cancer. In the study, a cohort of 398 cancer tissues from stage I to IV gastric cancer patients were assessed for programmed cell death 1 ligand 1 (PD-L1) expression and tumor-infiltrating lymphocyte (TIL) infiltration using immunohistochemistry to ascertain their survival correlation. The data revealed that higher TIL density correlated with less risk of disease progression, and exhibited survival benefits in gastric cancer patients, and PD-L1 positivity showed a significant association with the presence of high TIL infiltration. Furthermore, real-time quantitative polymerase chain reaction was performed to detect expression of multiple immune checkpoints with the relation to clinical outcome in 139 samples randomly selected from the same cohort, and higher messenger RNA levels of most immune checkpoints were associated with favorable outcome, while consistently showing a positive correlation with interferon gamma levels. In situ hybridization was used to determine the localization of Epstein–Barr virus (EBV) in 97 specimens, and showed EBV-positive gastric cancer samples correlated with PD-L1 expression and increased TIL density. These results suggest that induction of immune checkpoint within gastric cancer patients reflects a high immune infiltration density, especially in those with EBV-associated gastric cancer, which may direct patient selection for checkpoint blockade therapy.

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1. Introduction

The critical role of the immune microenvironment in tumor biology has been increasingly appreciated in recent years. Despite the rapid immune reaction triggered upon tumor surveillance, tumors frequently continue to develop and progress (June, 2007). Impediments of insufficient antigen presentation, lack of co-stimulating signal pathways, and aberrant inducement of co-inhibitory molecules are the main factors contributing to immune evasion by the tumor. Recently, blockade of immune checkpoints, such as anti-programmed death 1/programmed cell death 1 ligand 1 (anti-PD-1/PD-L1), has achieved objective responses with manageable adverse events in diverse tumors, including advanced melanoma, non-small-cell lung cancer (NSCLC), renal cell cancer, and ovarian cancer (Brahmer et al., 2012; Topalian et al., 2012). The exploration of immune-based biomarkers involved in tumor microenvironment is becoming a useful tool to predict patient outcomes and guide immunotherapy.

However, the intricate and dynamic immune contexture is heterogeneous among variant tumor types, and many studies on how it affects patient prognosis have produced conflicting results (Fridman et al., 2012). In the majority of cancers, it has been reported that immune checkpoints, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), PD-1/PD-L1, T-cell immunoglobulin domain and mucin domain 3 (TIM-3), indoleamine 2,3-dioxygenase (IDO), lymphocyte-activation gene 3 (LAG-3), interleukin-10 (IL-10), transforming growth factor-β (TGF-β), and forkhead box P3+ regulatory T (Foxp3 + Treg) cells, usually considered as mediating immune escape are characterized as having prognostic significance for patients with shorter survival (Giraldo et al., 2015; Jie et al., 2015; Komohara et al., 2015; Quereux et al., 2007; Wainwright et al., 2012). However, other studies on different tumors produced opposite results, challenging the concept of the relation of these immune inhibitory molecules to poor outcomes. In breast cancer, PD-L1 and IDO messenger RNA (mRNA) levels were proven to be associated with longer survival, and maintain independent favorable prognostic value in multivariate analysis (Jacquemier et al., 2012; Schalper et al., 2014). Overexpression of CTLA-4 was associated with favorable prognosis in patients with chronic lymphocytic leukemia (Joshi et al., 2007). High TGF-β mRNA levels were associated with better clinical outcome in a prognostic analysis of metastatic clear cell renal cell carcinoma (Busse et al., 2011). In addition, contrasting with most malignancies, a study showed that high Foxp3+ lymphocyte density in colorectal cancer tissue was associated with improved survival (Salama et al., 2009). Within the gastric cancer, previous studies also have conflicting results. Different from other three studies showing an association of PD-L1 positivity with poor patient outcome, Kim et al. demonstrated a favorable survival in PD-L1-positive cohort (Eto et al., 2015; Kim et al., 2016; Qing et al., 2015; Wu et al., 2006).

Owing to immune heterogeneity in dynamic tumor biology, it is crucial to define the roles of various immune-based factors in the development of histology-specific tumors. In this study, we reported the mRNA and protein levels of PD-L1 expression, tumor-infiltrating lymphocyte (TIL) infiltration status, and associated immune checkpoints within immune contexture of gastric cancer to assess their relations to prognostic significance, as well as the association of the presence of Epstein–Barr virus (EBV) infection with PD-L1 expression and TIL density in gastric cancer patients. Our study is broadening current observations of respective significance for immune-based markers in specific tumor type by exploring multicomponent panel of immune checkpoints to ascertain their concomitant prognostic effects within the same cohort of gastric cancer.

2. Materials and methods

2.1. Patients

A cohort of 444 archival formalin-fixed, paraffin-embedded (FFPE) gastric cancer tissues were collected between October 2007 and February 2010 at the Fudan University Shanghai Cancer Center (Shanghai, China) from stage I to IV gastric cancer patients after gastric resection and with no prior chemo- or radiotherapy. Each patient gave written informed consent and the study was approved and conducted in compliance with the Shanghai Cancer Center Institutional Ethics Committee. Median follow-up was 61.2 months (range, 12.2–79.9 months). At the time of last follow-up, 53.3% of patients with gastric cancer ($n = 212$) were alive. Information on the use of adjuvant chemotherapy was obtained from institutional medical records. Other details on patients' demographic characteristics are provided in Supplementary Table S1 (including 5-year overall survival (OS) association).

2.2. Immunohistochemical staining and histopathologic analysis

Serial FFPE tissue sections of 3-μm thickness were stained using autostainer Plus Link 48 (Dako, Glostrup, Denmark). The tissue sections were mounted on glass slides, dewaxed and rehydrated using xylene and graded alcohol washes, and antigen retrieval and deparaffinization were carried out using the EnVision™ FLEX Target Retrieval Solutions (Dako). Endogenous peroxidase activity was blocked by EnVision™ FLEX Peroxidase-Blocking Reagent (Dako). PD-L1 staining was applied with the primary antibodies (MKP1A07310, in-house-generated rabbit mAb clone from Merck-Serono [Darmstadt, Germany]) according to the manufacturer's instructions. After staining, the sections were dehydrated through ascending alcohols to xylene and mounted. Human tonsil and muscle tissue served as positive and negative control tissues, respectively (Supplemental Figure S1). The total number of evaluable gastric cancer samples was 398. Specimens with ≥5% membranous expression on tumor cells with ≥1 + intensity were considered PD-L1 positive.

The degree of infiltration by TILs was evaluated from 398 matched hematoxylin and eosin-stained specimens and assigned a semiquantitative score from 0 to 3: 0 = "none"; 1 = "rare"; 2 = "moderate and focal infiltration"; and 3 = "prominent and diffuse infiltration (high)". Graphic views

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