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Prognostic implication of programmed cell death 1 protein and its ligand expressions in endometrial cancer

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HIGHLIGHTS

- PD-L1 IHC was evaluated in relation to the tumor microenvironment in EC.
- High IC-PD-L1 expression was an independent adverse prognostic factor in EC.
- Combination of PD-L1 IHC with CD8 or PD-1 IHC conferred prognostic significance.
- Compartmentalized analysis of PD-L1 expression conferred prognostic significance.

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ABSTRACT

Objective. Monoclonal antibodies targeting programmed cell death-1 (PD-1)/programmed death ligand 1 (PD-L1) demonstrated promising clinical response. The predictive/prognostic value of PD-1/PD-L1 immunohistochemistry (IHC) has been evaluated in many cancer types. However, the prognostic value of PD-1/PD-L1 IHC has not been evaluated in endometrial cancer.

Methods. We conducted a retrospective study to quantify the IHC CD8, PD-1, and PD-L1 expressions in immune cells at center of tumor (CT), invasive margin (IM), and/or tumor cell in 183 primary endometrial cancer samples from a single cohort, followed by their reciprocal combinations, including compartmental differences, and correlated them with overall survival (OS) and progression-free survival (PFS).

Results. In repeated Cox multivariable models adjusted by clinicoimmunopathologic factors, high CT-PD-L1 was an independent adverse prognostic factor for PFS in all patients and in the microsatellite-stable subgroup. Immune marker ratios revealed independently shorter PFS for high CT-PD-L1/CT-CD8 and CT-PD-L1/CT-PD-1 ratios. Classification of endometrial cancer into four groups based on CT-CD8 and CT-PD-L1 revealed significantly different survival among groups.

Conclusions. The high PD-L1/CD8 ratio and the high expression of PD-L1 on immune cells were independent poor prognostic factors for PFS in endometrial cancer, providing insights into the tumor microenvironment.

1. Introduction

Endometrial cancer is the most frequently encountered gynecologic malignancy in developed countries, with approximately 320,000 women diagnosed annually, and is an important cause of mortality worldwide [1]. Despite its sharply increasing incidence, the optimal treatment for advanced endometrial cancer remains to be elucidated with a lack of approved targeted therapies and poor outcomes [2,3].

Recently, tumor microenvironment (TME) has gained focus in the development of cancer therapeutics [4]. Programmed cell death-1 (PD-1), an immune checkpoint receptor belonging to the CD28 family of T-cell receptors, and its ligand programmed death ligand 1 (PD-L1) are activated in TME contributing to antineoplastic immune evasion [4]. Monoclonal antibodies targeting the PD-1/PD-L1 pathway have shown promising clinical response in non-small-cell lung cancer (NSCLC), renal cell cancer (RCC), and mismatch repair (MMR)-deficient colorectal cancer (CRC) [5–7]. The immune checkpoint inhibitors induce

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T-cell activity recovery unleashing antineoplastic immune responses [4,8].

Endometrial cancer is considered as highly immunogenic with high PD-L1 expression [9], and there are a few ongoing clinical trials focusing on the PD-1/PD-L1 pathway in this type of cancer [10,11]. In endometrial cancer, MMR-deficient tumors and exonuclease domain of polymerase ε-mutated tumors have been suggested as candidates for immunotherapy because these tumors harbor not only increased tumor-specific neoantigens and $CD3^+/CD8^+$ tumor-infiltrating lymphocytes (TILs) but also frequent PD-1/PD-L1 expression in tumor cells (TCs) and immune cells (ICs) [12]. A clinical trial (NCT01876511) using anti-PD-1 antibody (pembrolizumab) in nine patients with MMR-deficient endometrial cancer validated that MMR status predicts a clinical benefit for the immune checkpoint inhibitor [6,13].

Although an increased $CD8^+$TIL has shown an association with improved prognosis in endometrial cancer, the value of PD-1 and PD-L1 immunohistochemistry (IHC) as prognostic markers has yet to be validated in endometrial cancer [14,15]. In addition, previous reports have shown that the relative avidity of distinct IC types affects patient outcomes through unique TME, emphasizing the need for combined analysis of immune markers [16–18]. Therefore, the aim of this study is to investigate CD8, PD-1, and PD-L1 expressions, and their different combinations, and to correlate them with clinicopathologic features and prognosis in patients with endometrial cancer. This study would shed light on the TME and the natural course of endometrial cancer, directing the development of optimal targeted therapy for patients with advanced disease.

2. Materials and methods

2.1. Tumor samples

A retrospective study was conducted in a consecutive series of 183 patients with primary endometrial cancer, including 168 endometrioid and 15 non-endometrioid histotypes (4 serous, 4 clear cell, 4 mixed, and 3 malignant mixed Müllerian tumor), who were diagnosed between March 2007 and January 2017 and had adequate tumor samples for analysis. Formalin-fixed, paraffin-embedded (FFPE) blocks from hysterectomy or curettage were retrieved from surgical pathology archives and partly from Korea Gynecologic Cancer Bank through the Bio & Medical Technology Development Program of the MEST, Korea (http://www.kgcb.or.kr) following the approval from the institutional review board of Gangnam Severance Hospital, Yonsei University, Seoul, Korea. Clinicopathologic features and survival data were obtained from a retrospective review of electronic medical records.

2.2. Immunohistochemistry

Following the review of hematoxylin and eosin slides of each case by two pathologists (SWH and JSK) to confirm the diagnosis of endometrial cancer, one representative FFPE block was chosen and serially sectioned into 4-μm thick slices for subsequent IHC. Then, the serial sections were stained for CD8, PD-1, PD-L1, and MMR proteins (MLH1, MSH2, MSH6, and PMS2) at the IHC surgical pathology laboratory. The staining details are described in Table S1. Microsatellite instability (MSI) status was determined by MMR IHC. Loss of MMR proteins was defined as complete loss of nuclear staining in the tumor cells in the presence of positive internal controls (stromal cells, lymphocytes, or normal endometrium).

2.3. Immunohistochemical analysis

The immunohistochemically stained whole-slide sections were numerically quantified for ICs in two distinct histologic compartments, designated as the center of tumor (CT) and invasive margin (IM) [19,20] and/or for TC, by a blinded pathologist (JSK) under the supervision of an experienced pathologist (SWH) in high-power fields (HPFs: 0.237 mm^2/HPF; Olympus BX51 microscope). CD8 density was evaluated as the number of CD8-positive T lymphocytes in 10 randomly selected HPFs. PD-1 expression was estimated as the percentage of PD-1-positive ICs relative to the total number of ICs in each compartment. Its expression was estimated as the percentage of TCs showing membranous PD-L1 staining and as the percentage of PD-L1-positive ICs showing cytoplasmic or membranous staining relative to the total number of ICs occupying each compartment was recorded for TCs and ICs, respectively. Subsequently, the ratios of CT-PD-1/CT-CD8, CT-PD-L1/CT-CD8, CT-PD-L1/CT-PD-1, and CT-PD-L1/IM-PD-L1 were calculated.

2.4. Statistical analysis

Descriptive statistics of baseline characteristics was expressed as mean ± SD (minimum–maximum) for continuous variables and frequency (percentage) for categorical variables.

To investigate the relationship between immune marker expression and survival, the IHC expression was subdivided into low and high levels using optimal cut-off points determined by the K-Adaptive partitioning method [21], which calculates the maximizing chi-square statistic based on log-rank statistics and estimates the best cut-off value. The cut-off values are shown in Table S2. The results of IHC were correlated with the patients' clinicopathologic features and survival.

To confirm the significant difference of overall survival (OS) and progression-free survival (PFS) rates based on immune markers' expression or ratio, survival curves were estimated using the Kaplan–Meier product limit method, and the OS and PFS rates were compared across the optimal cut-off point groups using the log-rank test.

Univariable Cox proportional hazards regression analysis was used to identify the remarkable immune markers for OS and PFS rates. In particular, marker combinations were created by using remarkable single immune markers. To handle type I error from multiple comparisons, false discovery rate (FDR) correction was applied. To identify remarkable immune markers, multivariable Cox proportional hazards regression analysis was conducted. For this analysis, immune markers with FDR-adjusted p-value (P_{FDR}) < 0.05 on univariable analysis and clinically important variables were entered.

All statistical tests were two-tailed, where $p < 0.05$ was considered statistically significant and $0.05 \le p < 0.15$ was considered to have a trend toward significance to increase the sensitivity to potential selection bias. Statistical analyses were performed using SAS version 9.3 (SAS Inc., Cary, NC, USA) and R version (package) 3.2.5. (survival and kaps package).

3. Results

3.1. Characteristics of the study population and immune markers

The clinicopathologic characteristics of the patients are summarized in Table S3. The mean age of patients was 53.0 ± 10.4 years. The median follow-up duration was 30.3 months. The immune marker expression profile including marker combinations and ratios and the illustrations are shown in Fig. 1 and Table S2. High CT-PD-1 and TC-PD-L1 expressions were correlated with only high levels of MSI (MSI-H). High CT-PD-L1 expression was correlated with post-menopause, high histologic grade (grade 3), deep myometrial invasion (≥1/2), lymphovascular invasion, adjuvant therapy, and MSI-H.

3.2. Impacts of TME on clinical outcomes

The results of the univariable survival analysis for CD8, PD-1, and PD-L1 expressions in all patients and in the MSS subgroup are presented in Table 1, Fig. 2, and Table S4. The independent prognostic implications for immune markers from the multivariable Cox proportional hazards

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