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Correlation between estrogen receptor and programmed death ligand-1 in type I endometrial cancer

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ABSTRACT

Objective: To determine the effect of estrogen receptor (ER) on programmed death-ligand 1 (PD-L1) expression in type I endometrial cancer (EC).
Material and Methods: This retrospective study included 85 patients with type I EC who underwent surgery at Dr. Soetomo Hospital between 2018 and 2022. A random sampling technique was employed. Immunohistochemistry (IHC) with ER and PD-L1 antibodies was performed on all samples. In this study, ER expression served as the independent variable, while PD-L1 expression was considered the dependent variable. Data analysis was performed using Spearman's rank correlation coefficient test.
Results: Out of the 85 patients with type I EC, 58 (68.2%) exhibited positive and 27 (31.8%) exhibited negative ER expression. Meanwhile positive PD-L1 expression was seen in 67 (78.8%) and 18 (21.2%) exhibited negative PD-L1 expression. The study revealed a strong negative correlation between ER and PD-L1 expression in EC (rho value = −0.886, p-value = 0.0001).
Conclusion: ER downregulates PD-L1 in type I EC. The findings of this study can be used as reference data and as the basis for further research, especially investigations of the prognostic and immunotherapeutic value of ER and PD-L1 expression in type I EC.

1. Introduction

Endometrial cancer encompasses a group of primary malignant epithelial tumors that originate in the inner surface of the uterine wall, known as the endometrium. The most common causes of EC include a family history of the disease, menstrual abnormalities, infertility, exposure to estrogen, the use of hormonal drugs, obesity, diabetes, and a high body mass index (BMI) [1]. Notably, the incidence of endometrial cancer is on the rise, particularly in developed countries, like the United States [2]. In Korea, the increasing incidence can be attributed to shifting lifestyles and environmental factors [3–5]. In Indonesia, EC is now the third leading cause of cancer-related death among women [6]. Projections suggest that the incidence of EC may increase by 20.3% by 2025, with a corresponding 17.4% rise in the number of deaths compared to 2018 [5].

Endometrial cancer is classified into two main histological types: I and II. Type I, which accounts for approximately 85% of cases of EC, is often linked to estrogen exposure. It is characterized by well-differentiated endometrioid histology, hormone receptor expression, and diploidy. Notably, it tends diagnosed at an early stages and carries a good prognosis. In contrast, Type II endometrial cancer is associated with advanced disease stages, non-endometrioid histological characteristics (serous endometrial carcinoma, clear cell carcinoma, or mixed carcinoma), high histological grade, aneuploidy, lack of hormone receptors, frequent TP53 mutations, and poor prognosis [7–9]. Recent advancements in genome analysis technology have revealed genomic anomalies within EC. Additionally, integrated genomic analyses have identified molecular subgroups that align with prognosis. The most

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notable approach of integrating molecular characteristics with EC classification by the Cancer Genome Atlas (TCGA) has resolved the numerous limitations in risk stratification [1]. The TCGA classifies EC into four distinct genomic categories that are Poymerase e mutation (POLE-Mutant), Mismatch Repair Deficiency (MMRd), P53 wild type / Non Specified Molecular Profile (NSMP), P53 high copy (P53-abn) [10].

Diverse biological abnormal changes in pathways have been discerned in EC cells. This has prompted the active development of novel therapeutic drugs and biomarkers, including immunomodulation inhibitors targeting programmed cell death protein 1 (PD-1) or PD-L1, to address these anomalies [1]. The landscape of cancer therapy has shifted with the advent of precision therapy and ongoing EC research. Immunotherapy, particularly is the use of a PD-1 inhibitors can be influenced by the expression of its ligand, PD-1 inhibitor as a potential treatment for EC the recommendations of the National Comprehensive Cancer Network (NCCN). Elevated expression of PD-L1 may be an immune response to tumor invasion.

Previous studies examining the correlation between ER and PD-L1 in breast cancer patients [11] have revealed that ER downregulates PD-L1 expression through interleukin-17 (IL-17). Based on these findings, we have designed a study to determine the effect of the ER on PD-L1 expression in type I EC wich is also estrogen dependent cancer, while also exploring the potential utility of ER status in the use of inhibitor immunotherapy in EC.

2. Materials and methods

This was a retrospective, cross-sectional histopathological study conducted using paraffin blocks of tissue obtained from patients who received treatment for type 1 EC at Dr. Soetomo Hospital Surabaya, Indonesia, between January 2018 to December 2022. We found total sample of 115 patient who meet inclusion and exclusion criteria, and 85 patient samples were collected through random sampling to reduce of bias. The inclusion criteria stipulated that patients had type I EC and underwent surgery at Dr. Soetomo Hospital, Surabaya, from 2018 to 2022. Paraffin blocks containing representative tumor masses were available at the Anatomical Pathology Laboratory of Dr. Soetomo Hospital. The exclusion criteria involved cases of type I EC that had malignancy in other organs. In this study, ER expression in type I EC served as the independent variable, and PD-L1 expression in type I EC was the dependent variable. Data analysis was performed using Spearman's rank correlation coefficient test.

Paraffin blocks of tissues from patients with type I EC were collected from the anatomical pathology laboratory to obtain immunohistochemical data for ER and PD-L1 antibodies. ER expression was determined, by staining endometrial tissue paraffin blocks with Biocare Medical ER™ (SP1) ER antibodies using the LSAB II method and fixation with 10% neutral buffered formalin (NFB). For PD-L1 expression analysis, immunohistochemical staining was performed on endometrial tissue paraffin blocks using the PD-L1 GeneAb™ antibody from GenomeMe™ clone IHC411. It was derived from the membrane or cytoplasm of rabbit monoclonal cells using the LSAB II method and fixation with 10% NFB. The methodology was approved by the Research Ethics Committee of Dr. Soetomo Hospital Surabaya, Indonesia.

3. Results

A total of 85 patient samples were collected. Results showed that there were 41 patients (48.2%) in the age group $<$ 55 years and 44 patients (51.8%) in the age group $>$ 55 years. The BMI group had 6 patients (7.1%) in the underweight group, 36 patients (42.4%) in the normal-weight group, 14 patients (16.5%) in the overweight group, 23 patients (27.1%) in the obesity class I group, and 6 patients (7.1%) in the obesity class II group. In the menopausal status group, 39 patients (45.9%) were in the premenopausal group and 46 patients (54.1%) in the menopausal group. The disease stage group had 45 patients (52.9%) in the early stage group and 40 patients (47.1%) in the advanced stage group. The cell differentiation group, 54 patients (63.5%) in the low-grade group, and 31 patients (36.5%) in the high-grade group. The nodal metastasis group had 76 patients (89.4%) in the group with no nodal metastasis and 9 patients (10.6%) in the group with nodal metastatic. In the LVSI group, 59 patients (69.4%) were found in the group with no LVSI and 26 patients (30.6%) in the group with LVSI. The myometrial invasion group had 22 patients (25.9%) in the group with $<$ 1/2 myometrial invasion, and 63 patients (74.1%) in the group with $\geq$ 1/2 myometrial invasion. The adjuvant therapy group had 19 patients (22.4%) in the group that was not given adjuvant therapy, and 66 patients (77.6%) in the group that was given adjuvant therapy. Table 1.

3.1. Positive ER and PD-L1 expression in type I EC

Among the 85 patients with type I EC, 58 (68.2%) exhibited positive ER expression, while 27 (31.8%) were ER-negative. Moreover, out of the

Table 1
Sample Demographic.

Characteristics	Frequency	Percentage
Age	Mean: 53.42	
<55 years	41	48.2 %
≥55 years	44	51.8 %
Sum	85	100 %
BMI		
Underweight	6	7.1 %
Normoweight	36	42.4 %
Overweight	14	16.5 %
Obesity class 1	23	27.1 %
Obesity class 2	6	7.1 %
Obesity class 3	0	0 %
Sum	85	100 %
Menopause status		
Yes	46	54.1 %
No	39	45.9 %
Sum	85	100 %
Cancer Stage		
Early Stage (I, II)	45	52.9 %
Advanced Stage (III, IV)	40	47.1 %
Sum	85	100 %
Myometrium Invasion:		
<1/2 myometrium	22	25.9 %
≥1/2 myometrium	63	74.1 %
Sum	85	100 %
Nodal metastasis:		
Yes	9	10.6 %
No	76	89.4 %
Sum	85	100 %
Cell Differentiation (*tumor grade*):		
Low grade (I and II)	54	63.5 %
High grade (III and IV)	31	36.5 %
Sum	85	100 %
LVSI:		
Yes	26	30.6 %
No	59	69.4 %
Sum	85	100 %
Adjuvant Therapy:		
Yes	66	77.6 %
No	19	22.4 %
Sum	85	100 %
Expression of ER:		
Positive	58	68.2 %
Negative	27	31.8 %
Sum	85	100 %
Expression of PD-L1:		
Positive	67	21.2%
Negative	18	21.2 %
Sum	85	100 %

85 patients with type I EC, 67 (78.8%) displayed positive PD-L1 expression, whereas 18 (21.2%) were PD-L1-negative. (Fig. 1).

3.2. Correlation between ER and PD-L1 expression in type I EC

Coefficient correlation analysis using Spearman's rank was conducted to test the correlation between ER and PD-L1 expression. The results indicated a statistically strong negative correlation between ER and PD-L1 expression in type I EC, with a rho value of − 0.886 and a p-value of 0.0001. (Fig. 2) Based on this result we assume that ER expression downregulates PD-L1 in type I EC.

4. Discussion

The result of the present study found that ER expression was positive in 68.2% of the cases of type 1 endometrial cancer. This finding is similar to the results of the study by Wang et al. in 2007, in which ER positivity was found in 59.8% of cases of endometrial cancer in a study population in China [12]. The results of the present study also indicated that PD-L1 expression was positive in 78.8% of the cases of type 1 endometrial cancer. A meta-analysis of 11 studies revealed that PD-L1 expression in endometrial cancer is quite diverse. The results of our study are similar to those of Zhang et al. (2020), in which PD-L1 positivity was found in 70.14% of endometrial cancer cases in a study population in Japan [13]. Engerud et al. also reported PD-L1 positivity of 63% in primary tumors [14]. Through gene expression analysis, researchers have shown that PD-L1 is upregulated in PD-1-positive tumor cells [15]. In contrast, Pasenan et al., in a 2019 study of patients in Finland, reported a PD-L1 positivity of only 8.58%. This difference is likely due to racial differences in the research sample, but whether there is a relationship between PD-L1 expression and race requires further research [16].

4.1. Role of PD-1/PD-L1 in immunotherapy for EC

The PD-1/PD-L1 pathway plays a crucial role in the immune escape mechanism and growth of cancer cells in EC. Clinical trials investigating efficacy of PD-1/PD-L1 inhibitor have shown promising results in EC [17]. PD-1 inhibitors as a potential treatment for EC has been recommended by NCCN. Immunotherapy, particularly is the use of a PD-1 inhibitors can be influenced by the expression of its ligand, Elevated expression of PD-L1 may be an immune response to tumor invasion. Recent investigations have unveiled that anti-PD-1/PD-L1 first line therapy yields response rates varying between 20% and 65% in

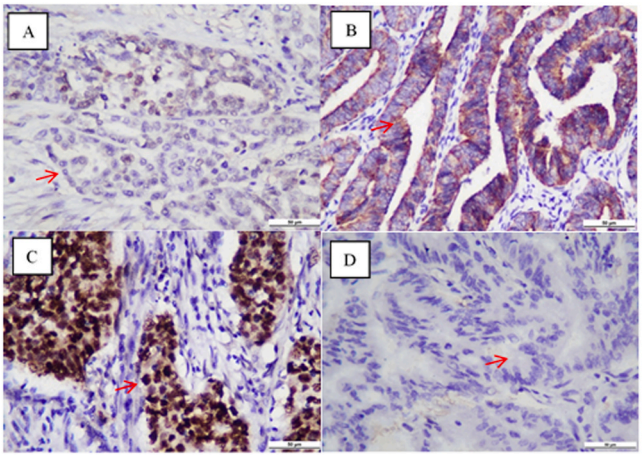

Fig. 1. Negative expression of ER in the cell nucleus (A). Strong expression of PD-L1 in the cell membrane (B). Strong expression of ER on the cell nucleus (C). Negative expression of PD-L1 on the cell membrane (D). There is a negative correlation between ER expression and PD-L1 expression. (magnification: 400x, scale bar: 50 μm).

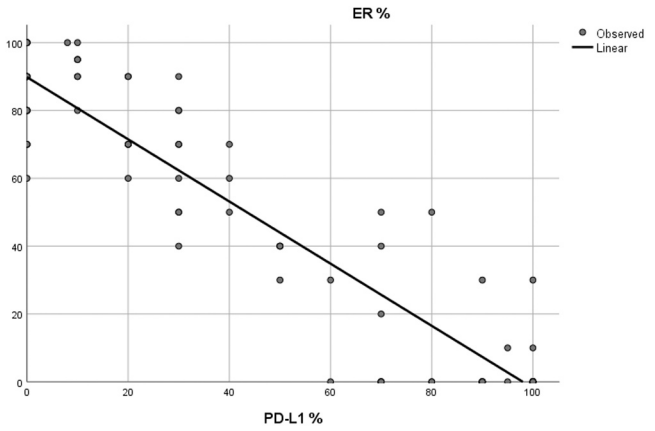

Fig. 2. Correlation Coefficient Curve of ER and PD-L1 Expression.

PD-L1-positive tumours in various cancers, including EC [17]. Conversely, tumours lacking PD-L1 expression exhibit response rates ranging from 0% to 17% across diverse tumour types [18]. The significance of PD-L1 expression within the tumour microenvironment is recognised as a pivotal biomarker for identifying individuals who are more likely to benefit therapeutically from immunotherapy.

4.2. PD-L1 regulation by estrogen pathway in cancer

Estrogens downregulate PD-L1 expression in EC and correlates with ER-negative status in EC [11] Estrogen mechanisms modifying PD-L1 seem to be complex and may depend on several factors such as cancer type, histology, tumor mutational burden (TMB), ER isoforms, Aromatase expression and estrogen levels. This relationship needs to be explored since E2 pathway blocking could improve immunotherapy in some cancers [19].

The results of the present study showed a strong negative correlation between ER and PD-L1 expression in type 1 endometrial cancer (rho value = −0.886 and p-value = 0.0001). This negative correlation may be because ER downregulates PD-L1 by activated estradiol to recruit a repressor of estrogen receptor activity (REA) and form the ER/REA complex, which binds the estrogen receptor element (ERE) on the retinoic acid receptor-related orphan nuclear receptor gamma (RORγT) promoter. As a result of the inhibition of RORγT, Th17 cell differentiation, and infiltration are impeded, thereby weakening IL-17 signal transduction intensity and decreasing PD-L1 expression [11,20].

ER deficiency causes increased infiltration of Th17 cells, which upregulate IL-17 signal transduction. IL-17 binds its receptor IL-17R in the tumor microenvironment, which activates NF-κB signaling and NF-κB translocation to promote PD-L1 translation and increase expression on the cell membrane. The synergy of Il-17, IFNγ, and TNFα promotes PD-L1 expression [11,20]. After translation, NfKB regulates and maintains PD-L1 expression by inducing the transcription of the COPS5 gene, which deubiquitinates PD-L1 protein to stabilize PD-L1 on the cell membrane [11,20] (Fig. 3).

To the best of our knowledge, the present study is the first in which the correlation between ER and PD-L1 expression has been confirmed in type I EC. New therapies for EC therapy are rapidly developing. One of these novel therapies is the use of PD-1 inhibitor, the effectiveness of this approach has been demonstrated by measuring the degree of ligand (PD-L1) expression in immune cells and tumor cells. Based on these results we assume that ER status can predict the response of PD-1 inhibitor in EC, and adding anti-estrogen could potentially improve the response of PD-1 inhibitor in EC. The limitations of this study, insofar as it was a retrospective study and did not address all the possible factors that may influence PD-L-1 expression. Notwithstanding these limitations, the findings we report here can serve as reference data or the base for further

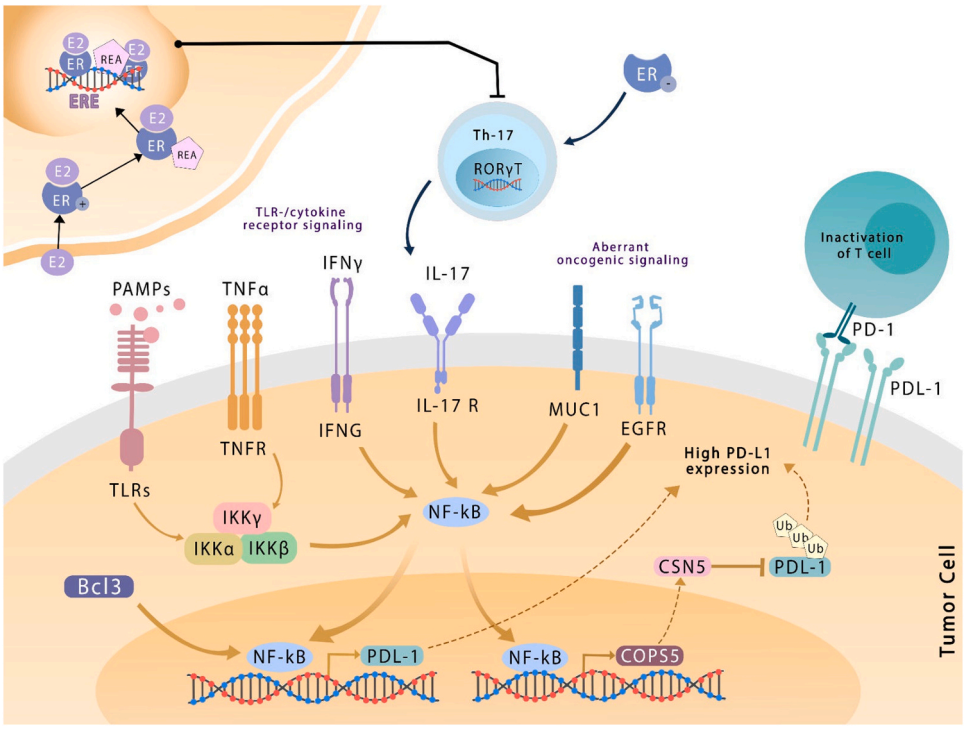

Fig. 3. PD-L1 regulation by estrogen pathway in cancer

research into the potential of ER and PD-L1 as prognostic and improve the efficacy of immunotherapy in EC.

5. Conclusion

ER downregulates PD-L1 in type 1 EC. The findings of this study can be used as reference data and as the basis for further research, especially investigations of the prognostic and immunotherapeutic value of ER and PD-L1 expression in type 1 EC. Further research is needed to determine the role of ER and PD-L1 expression in disease outcomes, recurrence rates and survival rates of patients with type 1 EC.

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CRediT authorship contribution statement

Setyo Teguh Waluyo: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Brahmana Askandar Tjokropra-wiro:** Formal analysis, Supervision, Writing – original draft. **Anny Setijo Rahaju:** Data curation, Writing – review & editing, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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