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Original contribution

Analysis of PD-L1 expression in trophoblastic tissues and tumors☆

Bingjian Lu MD[a,b,*], Xiaodong Teng MD[c], Guoxiang Fu MD[d], Lei Bao MD[e], Jinglong Tang MD[f], Haiyan Shi MD[a], Weiguo Lu MD[b,g], Yan Lu PhD[b,h,]**

[a]*Department of Surgical Pathology, Women's Hospital, School of Medicine, Zhejiang University, Zhejiang Province 310006, China*
[b]*Center for Uterine Cancer Diagnosis and Therapy of Zhejiang Province, Women's Hospital, School of Medicine, Zhejiang University, Zhejiang Province 310006, China*
[c]*Department of Surgical Pathology, the Affiliated First Hospital, School of Medicine, Zhejiang University, Zhejiang Province 310006, China*
[d]*Department of Surgical Pathology, the Affiliated Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Zhejiang Province 310006, China*
[e]*Department of Surgical Pathology, Shaoxing Women's Hospital, Shaoxing, Zhejiang Province 3123000, China*
[f]*Department of Surgical Pathology, the Affiliated Second Hospital, School of Medicine, Zhejiang University, Zhejiang Province 310006, China*
[g]*Department of Gynecologic Oncology, the Affiliated Women's Hospital, School of Medicine, Zhejiang University, Zhejiang Province 310006, China*
[h]*The Institute of Translational Medicine, School of Medicine, Zhejiang University, Zhejiang Province 310006, China*

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Summary The immune checkpoint proteins, programmed death receptor 1 (PD-1) and programmed death ligand 1 (PD-L1), are crucial for maintaining fetomaternal immune tolerance and immune escape in cancers. In this study, we performed a comprehensive immunohistochemical study of PD-L1 expression in a large cohort of trophoblastic tissues and tumors. We found that normal villi and hydatidiform moles showed a heterogeneous PD-L1 staining among trophoblast (strong in syncytiotrophoblast, moderate in intermediate trophoblast, and weak/negative in cytotrophoblast). Eleven exaggerated placental sites (100%) showed variable PD-L1 staining, whereas 7 (36.8%) of 19 placental site nodules/plaques were weakly positive for PD-L1 ($P < .001$). All gestational choriocarcinomas (CCs; n = 63), epithelioid trophoblastic tumors (n = 12), and placental site trophoblastic tumors (n = 41) were PD-L1 positive, with most showing strong staining. However, PD-L1 expression was lower in epithelioid trophoblastic tumors compared with placental site trophoblastic tumors and CCs ($P = .004$). Three presumably germ cell–derived pure CCs, the CC elements in 13 mixed germ cell tumors, and 4 gastric/rectal CCs were also positive for PD-L1, with widespread staining. The background nontrophoblastic tissues, such as endometrial glands, squamous cells, and adenocarcinomas, were PD-L1 negative. Western blot analysis showed that PD-L1 was expressed in all 3 trophoblastic cell lines. We conclude that PD-L1 is a sensitive but nonspecific marker for trophoblast and related tumors.

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* Correspondence to: B. Lu, Department of Surgical Pathology, the Affiliated Women's Hospital, School of Medicine, Zhejiang University, Zhejiang Province 310006, China.
** Correspondence to: Y. Lu, The Institute of Translational Medicine, School of Medicine, Zhejiang University, Zhejiang Province 310006, China.
E-mail addresses: lbj@zju.edu.cn (B. Lu), yanlu76@zju.edu.cn (Y. Lu).

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The frequent strong PD-L1 expression suggests that immune checkpoint blockade could be a promising approach in treating trophoblastic tumors that merits further investigation.

1. Introduction

Gestational trophoblastic disease is uncommon. It has a range of entities including hydatidiform moles (HMs) and gestational trophoblastic neoplasms (GTN). Moles can potentially spread to distant sites, and up to 10% of cases can develop into GTN [1]. More than 90% of GTN patients can be cured by chemotherapy; however, some patients, particularly those with placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT), are insensitive to chemotherapy and may have fatal clinical courses [2,3]. The trophoblastic neoplasms, predominantly choriocarcinomas (CC), can also be derived from germ cells or somatic cells [4-6]. Patients with CC dedifferentiated from gastrointestinal adenocarcinomas have an aggressive clinical course, with most patients dying within 1 year [6]. The differential diagnosis between trophoblastic tumors and morphologic mimics will sometimes be difficult [7-9]. There is a permanent need to find diagnostic and therapeutic targets for trophoblastic tumors.

Programmed death receptor 1 (PD-1; CD279) and its ligands, programmed death ligands 1 (PD-L1; B7-H1, CD274) and 2 (PD-L2; B7-DC, CD273), are the most studied immune checkpoint proteins; these are crucial for maintaining immune self-tolerance and facilitating cancer cells to escape immune surveillance [10]. PD-1 is expressed on the surface of tumor-infiltrating lymphocytes (mainly CD4+ T cells), B cells, natural killer cells, and macrophages; PD-L1 seems to be frequently upregulated in a subset of human solid tumors, such as non–small cell lung cancers and colorectal cancers [11,12]. Anti–PD-1 or anti–PD-L1 agents, such as nivolumab and pembrolizumab, have been validated as beneficial for patients with various advanced cancers [13]. Therefore, PD-L1 expression in cancer tissues has been described as a useful indicator for anti–PD-1/PD-L1 immunotherapy [14].

It has been indicated that PD-L1 plays an essential role in fetomaternal tolerance [15]. PD-L1 can be expressed in cytotrophoblast and syncytiotrophoblast, the fetal cells at the fetomaternal interface [16], and in a small number of trophoblastic tumors [17-19]. Nevertheless, the data on PD-L1 expression in trophoblastic tissues and tumors are very limited to date.

Prompted by these observations, we carried out a comprehensive immunohistochemical study of PD-L1 expression in a large cohort of trophoblastic tissues and tumors. The goals of our study were (1) to assess the potential value of PD-L1 immunostaining in the diagnosis of trophoblastic diseases and tumors and (2) to provide a rationale for future anti–PD-1/PD-L1 immunotherapy in trophoblastic tumors.

2. Materials and methods

2.1. Case selection

This study was approved by the hospital's institutional review board (No. 20170136). A total of 265 cases of pregnancy-related nonneoplastic tissues, trophoblastic tumors, and tumors with trophoblastic elements were obtained from the in-house or consultation files of our institutions. The patients' clinical information is shown in Table 1.

The nonmolar uterine conceptions/placentas included 10 first-trimester, 5 second-trimester, and 10 third-trimester placentas, as well as 5 tubal conceptions (4-6 gestational weeks). The 11 women with exaggerated placental site (EPS) had a median age of 35 years (range, 23-40 years). Five occurred after abortions/term pregnancies and 6 after a complete HM (CHM). Nineteen placental site nodules/plaques (PSNs) were incidentally found by uterine curettages in women aged 26 to 52 years. Thirty-nine patients with CHM were at 7 to 12 gestational weeks. Two high-risk patients received 6 courses of methotrexate (MTX) chemotherapy. The remaining 37 CHMs and 6 partial HMs (PHMs) were only regularly followed up by the clinicians. Eleven women with invasive HM (IHM) underwent total hysterectomy. Two had metastasis in the lung or the pelvis. Eight patients were further treated by 1 to 7 courses of chemotherapy (MTX, MTX + actinomycin D (ACTD), or Etoposide (VP-16), MTX, ACTD-Cyclophosphamide (CTX), Vincristine (VCR) (EMA-CO)). During the time of follow-up (12-24 months), all patients with moles had an unremarkable clinical course.

A total of 119 GTNs were recruited in this study. The prognostic factors, such as antecedent pregnancy, months from index pregnancy, pretreatment serum β-human chorionic gonadotropin, and sites of metastasis, were only available in 76 patients. The International Federation of Gynecology and Obstetrics (FIGO) prognostic score was applied to separate the patients into low-risk (≤6) and high-risk (≥7) categories [1]. The FIGO score and stage (2009) are given in Table 1. Eighteen CC, 41 PSTT, and 15 ETT patients underwent surgeries including hysterectomy, lung lobectomy, and abdominal mass excision. Sixty-three CC, 5 PSTT, and 11 ETT patients received 6 to 11 courses of chemotherapy. The regimens included EMA-CO, Etoposide (VP-16), Cisplatin-Etoposide (VP-16), MTX, ACTD, Cyclophosphamide (CTX), Vincristine (VCR) (EP-EMA), and Taxol, Cisplatin (TP), and others. Follow-up data were only available in 41 GTNs

Table 1 Clinical features of trophoblastic tissues and diseases

	Cases (male)	Age (y), median (range)	Sites (n)	FIGO stage [a] (n)	Prognostic score (n-LR; n-HR) [a]	Follow-up (n)
Trophoblastic tissues						
Placenta	30	29 (24-39)	Uterus (25), fallopian tube (5)			
Fetal membrane	10	29 (25-36)	Uterus (10)			
Gestational trophoblastic tumor–like lesions						
EPS	11	35 (23-40)	Uterus (11)			
PSN	19	31 (26-52)	Uterus (19)			
Molar pregnancy						
CHM	39	32 (21-54)	Uterus (39)			ANED (39)
PHM	6	28 (24-31)	Uterus (6)			ANED (6)
IHM	11	49 (30-53)	Uterus (11)	I (9), III (1), IV (1)	5; 6	ANED (11)
GTN						
Gestational CC	63	36 (19-61)	Uterus (46), fallopian tube (1), ovary (2), pelvis (3), lung (6), brain (5)	I (18), II (2), III (9), IV (6)	16; 19	ANED (13); DOD (2)
PSTT	41	31 (21-51)	Uterus (41)	I (27), II (2), III (1), IV (1)	27; 4	ANED (18); DOD (1)
ETT	15	40 (28-57)	Uterus (11), lung (3), abdominal wall (1)	I (5), II (1), III (4)	3; 7	ANED (5); AWD (1); DOD (1)
Germ cell–derived CC						
Pure CC	3 (1)	19 (12-35)	Ovary (2), mediastinum and lung (1)			ANED (2)
CC in MGCT	13 (6)	27 (5-36)	Ovary (7), testis (3), para-aortic lymph node abdominalis (2), retroperitoneum (1)			ANED (3)
Somatic cell–derived CC	4 (1)	68 (62-74)	Stomach (3), rectum (1)			

Abbreviations: ANED, alive with no evidence of disease; AWD, alive with disease; DOD, death of disease; HR, high risk; LR, low risk.

[a] The FIGO prognostic score and stage (2009) were given [1]. Patients were separated into low-risk (≤6) and high-risk (≥7) categories according to the prognostic scores.

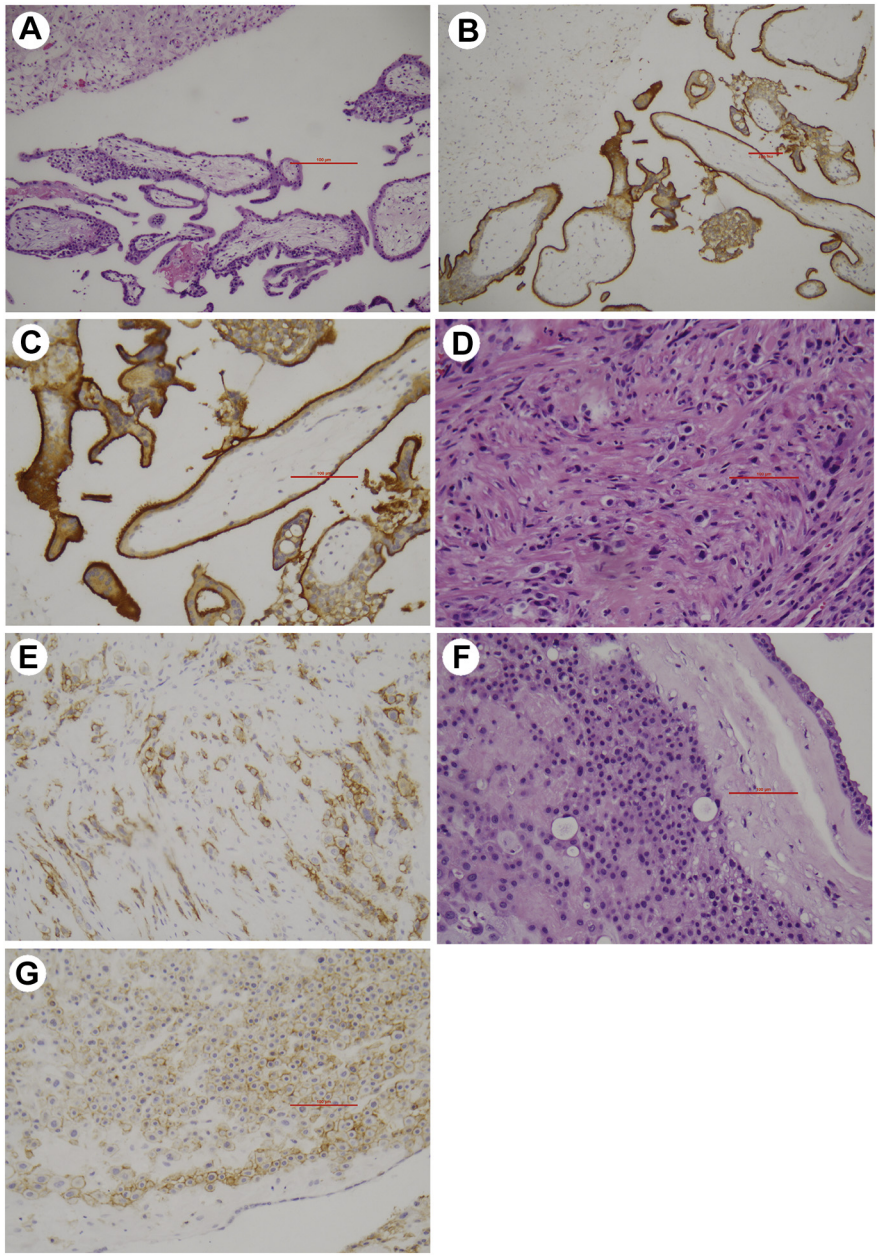

Fig. 1 PD-L1 expression in trophoblastic tissues. A, D, and F, Histology of the first-trimester nonmolar placenta, placental site, and the chorionic leave. PD-L1 expression is strong in the syncytiotrophoblast of the villi (B and C) and moderate in the intermediate trophoblast of placental site (E), and the chorionic laeve in the fetal membrane (G). Note the negative/weak staining in the cytotrophoblast. PD-L1 expression is absent in the nontrophoblastic background tissues, such as villous mesenchymal cells (B and C), deciduas (B), smooth muscle cells (E), amnion cells (G), and so on. H&E: A, D, and F. Immunohistochemistry: B, C, E, and G. Original magnifications ×10 (A and B; red scale, 100 μm) and ×20 (C-G; red scale, 100 μm).

(Table 1). Two CC, 1 PSTT, and 1 ETT patients died of disease at 7 to 28 months after their initial diagnosis.

Three pure CCs were presumed to be derived from germ cells. Two patients with pure ovarian CC were unmarried young girls at the ages of 12 and19 years. They had no history of pregnancy. Both patients were treated with unilateral salpingo-oophorectomy and chemotherapy. The remaining germ cell–derived CC was found in a 35-year man who had tumors in the mediastinum and right upper lobe of the lung. Seven patients with ovarian malignant mixed germ cell tumors (MGCTs) had a median age of 18 years (range, 5-36 years). The sites of 6 male MGCTs included the following: testis in 3 patients, para-aortic lymph node abdominalis in 2, and retroperitoneum in 1. The ages ranged in 22 to 35 years (median, 29.5 years). Three male patients with gastric CCs were 62, 74, and 76 years of age, respectively, whereas the rectal one was found in a 58-year-old woman. The clinical details of these patients were unavailable.

The archival hematoxylin-and-eosin (H&E) slides were reviewed by 2 gynecologic pathologists (L. B. and S. H.)

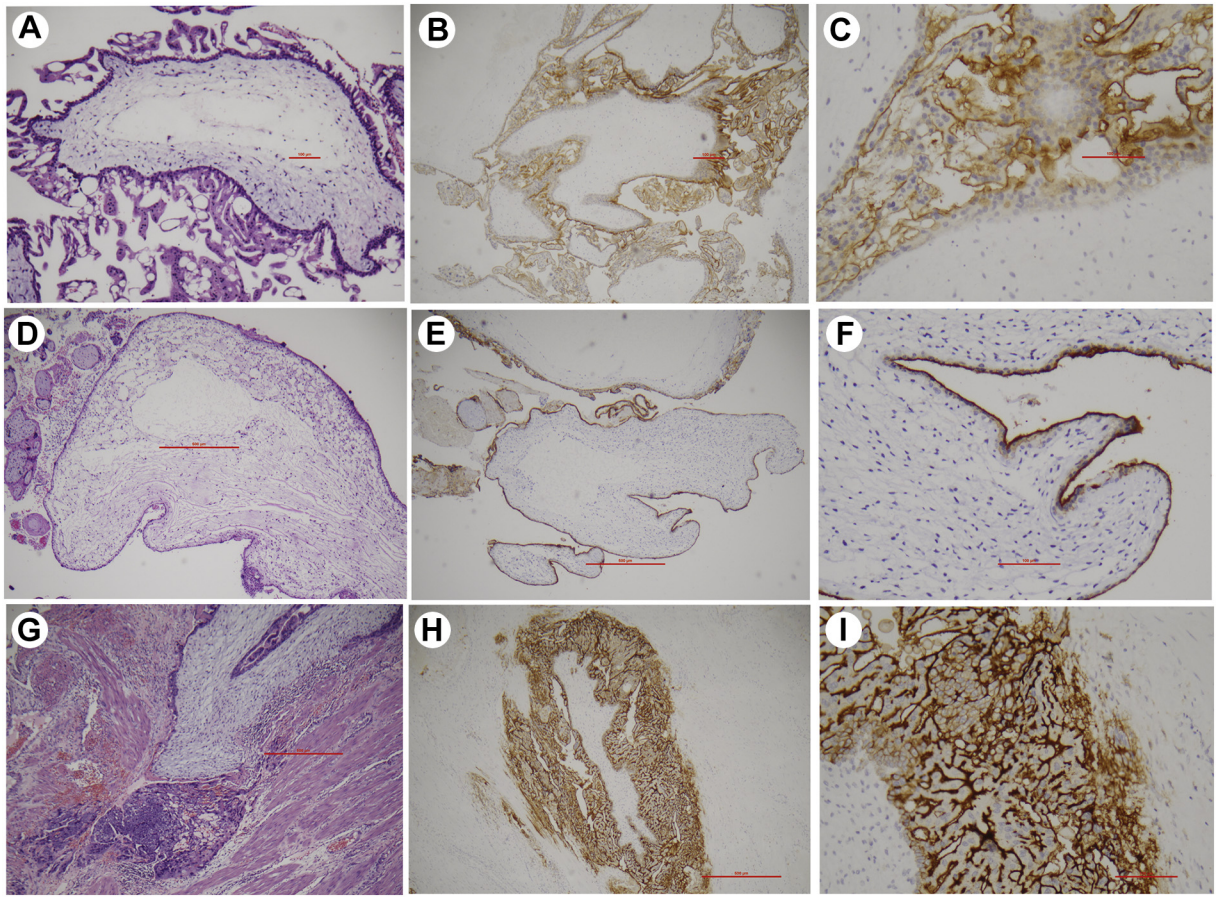

Fig. 2 PD-L1 expression in HM. Both complete (A-C) and PHMs (D-F) show strong membrane PD-L1 staining of syncytiotrophoblast, whereas IHMs with florid hyperplasia of trophoblast are diffusely and strongly positive for PD-L1 (G-I). H and I, The uterine myometrium is PDL-1 negative. H&E: A, D, and G. Immunohistochemistry: B, C, E, F, H, and I. Original magnifications ×5 (D and E; red scale, 500 μm), ×10 (A, B, G, and H; red scale, 100 μm), and ×20 (C, F, and H; red scale, 100 μm).

according to the recent World Health Organization classifications of the tumors of the female genital tract [1]. MGCT contained a variable component of CC (n = 13), embryonic carcinoma (n = 8), teratoma (n = 5), yolk sac tumor (n = 4), and dysgerminoma/seminoma (n = 3). One gastric or rectal CC had a substantial part of concurrent moderately to poorly differentiated adenocarcinoma. Immunohistochemistry and fluorescent in situ hybridization (confirming triploid in PHM) were used to aid in the diagnosis when necessary. The antibodies included p57, β-human chorionic gonadotropin, α-inhibin, human placental lactogen, CD146 (Mel-CAM), p63, GATA3, OCT4, SALL4, glypican 3, and so on.

2.2. Immunnohistochemistry

Whole tissue sections at 4 μm were used for immunohistochemistry. This was carried out using the mouse antihuman PD-L1 antibody (ab210931; Abcam, Shanghai, China) at a dilution of 1:100. A 2-step EnVision immunostaining procedure (Dako, Carpentaria, CA) was performed according to the manufacturer's protocols. Normal human placenta was used as a positive control, and PD-L1–negative cervical squamous cell carcinoma cases were used as a negative control. Membrane staining was defined as positive. The immunostaining was interpreted by 2 pathologists (L. B. and S. H.) with the assessment of both the percentage of positive cells (0, <5%; 1+, 5%-24%; 2+, 25%-49%; 3+, 50%-74%; 4+, ≥75%) and staining intensity (0, no staining; 1, weak; 2, moderate; 3, strong). A composite immunoscore (range, 0-12) was calculated by multiplying the scores of positive cells by the staining intensity. The composite score of 1 to 3 was defined as weak positive and 4 or higher as strongly positive.

2.3. Western blot analysis

Human placental trophoblastic cell line SWAN-71 and CC cell lines JEG-3 and JAR are permanently maintained in our laboratory (Center for Uterine Cancer Diagnosis and Therapy of Zhejiang Province, Women's Hospital, School of Medicine, Zhejiang University). SWAN-71 and JEG-3 were cultured in Eagle's minimum essential medium (Gibco, Grand Island, NY), and JAR was cultured in RPMI-

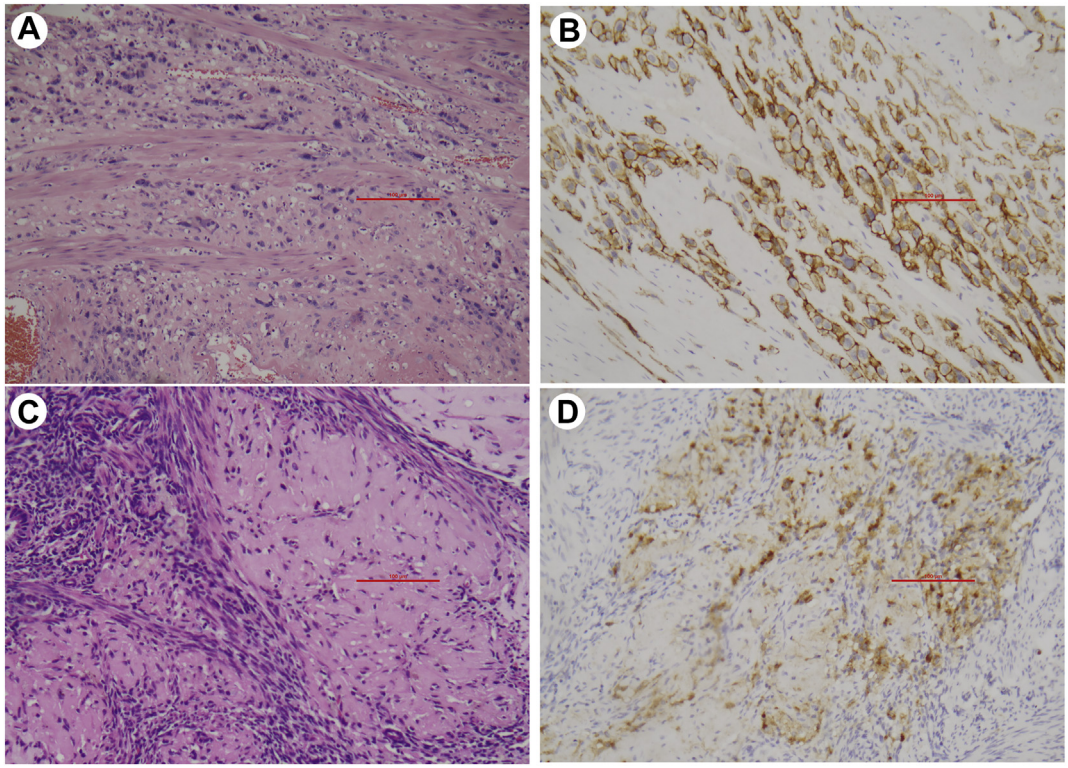

Fig. 3 PD-L1 expression in trophoblastic tumor–like lesions. EPSs show diffuse membrane PD-L1 staining (A and B), whereas PSNs exhibit focal membranous staining (C and D). D, However, cytoplasmic PD-L1 staining can be present in PSN. H&E: A and C. Immunohistochemistry: B and D. Original magnifications ×20 (red scale, 100 μm).

1640 medium supplemented with 10% (vol/vol) heat-inactivated bovine serum (Gibco). All cell lines were grown at 37°C in an atmosphere of 95% air and 5% CO_2. The mouse antihuman PD-L1 antibody, the same as that used for immunohistochemistry but at a dilution of 1:1000, and rabbit antihuman glyceraldehyde-3-phosphate dehydrogenase (GAPDH; ab9485; Abcam; dilution: 1:2000) were used in Western blot analysis in accordance with standard protocols. The human cervical cancer cell line HELA and the embryonic kidney cell line 293T were used as positive controls for PD-L1 expression.

Briefly, aliquots of 50-μg protein extracts were separated on an 8% sodium dodecyl sulfate–polyacrylamide gel according to the protein molecular weight. The protein was then electrophoretically transferred on to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). After blocking with TBS-Tween 20 (TBST) containing 5% nonfat milk, the membranes were incubated with primary antibody diluted to 1:1000 in TBST overnight at 4°C, followed by peroxidase-conjugated second antibody diluted to 1:5000 in TBST for 1 hour at room temperature. The blots were eventually developed with the ECL 2 Western Blotting Substrate (Thermo Scientific, Waltham, MA).

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 16.0 for Windows (SPSS, Chicago, IL). The difference in

Table 2 PD-L1 expression in trophoblastic tumors

	PD-L1–positive cases (strong positive)	Immunoscores of PD-L1 (mean ± SD)
GTN		
PSTT	41/41 (100%), 40/41 (97.6%)	9.8 ± 2.8
ETT	12/12 (100%), 10/12 (83.3%)	7.1 ± 3.0
Gestational CC	63/63 (100%), 60/63 (95.2%)	9.8 ± 2.9
Germ cell–derived CC		
Pure CC	3/3 (100%), 3/3 (100%)	10.7 ± 2.3
CC in MGCT	13/13 (100%), 13/13 (100%)	7.6 ± 2.3
Somatic cell–derived CC	4/4 (100%), 4/4 (100%)	8.0 ± 1.4

NOTE. A composite immunoscore was calculated by multiplying the scores of positive cells by the staining intensity. The composite score of 4 or higher was defined as strong positive.

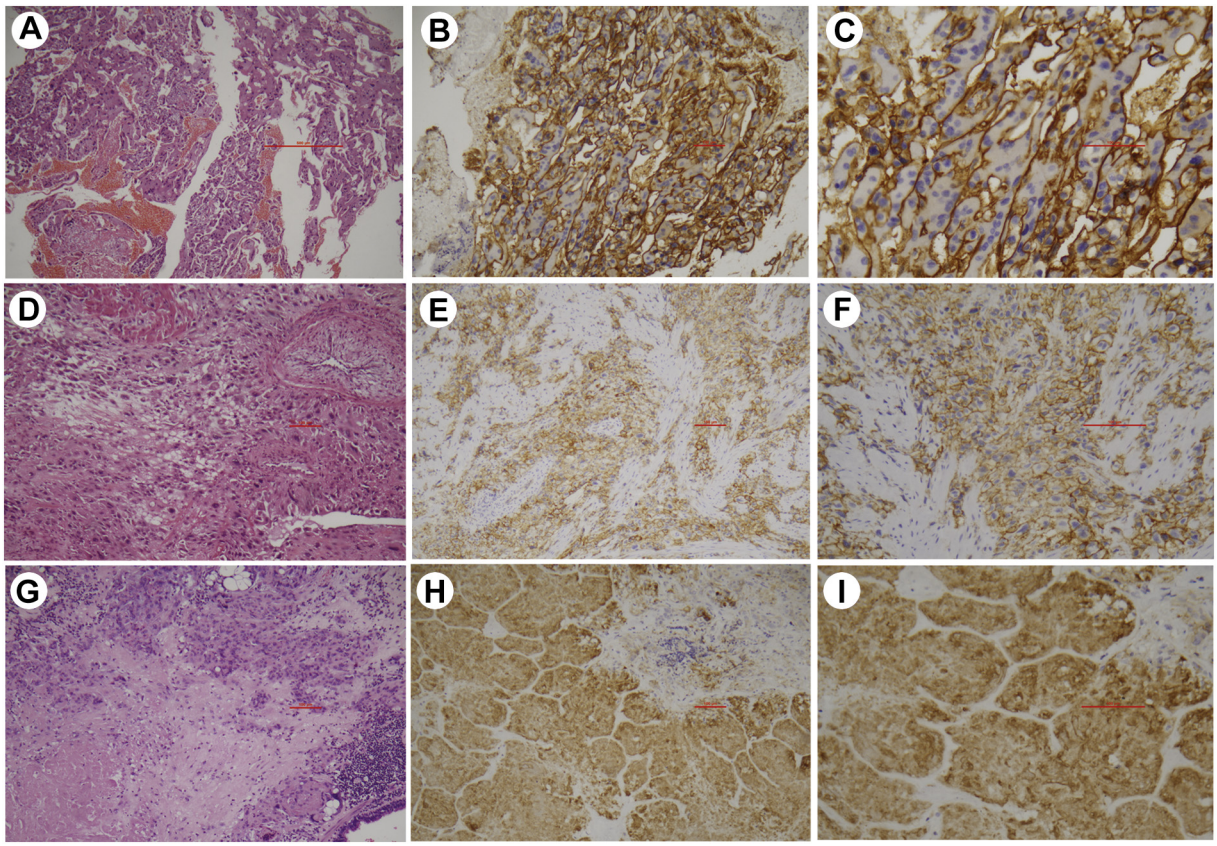

Fig. 4 PD-L1 expression in gestational trophoblastic tumors. Depicted is the characteristic histopathology of uterine CC (A), PSTT (D), and lung ETT (G). Diffuse and distinct membrane PD-L1 staining is found in CC (B and C), PSTT (F and G), and ETT (H and I). H&E: A, D, and G. Immunohistochemistry: B, C, E. F, H, and I. Original magnifications ×5 (A; red scale, 500 μm), ×10 (B, D, E, G, and H; red scale, 100 μm), and ×20 (C, F, and I; red scale, 100 μm).

composite immunoscores between different groups was analyzed using the independent-sample *t* test or 1-way analysis of variance (Kruskal-Wallis) test when necessary. The 2-sided $P < .05$ was considered statistically significant.

3. Results

3.1. PD-L1 expression in trophoblastic tissues

The placentas at various trimesters or between uterine and tubal pregnancies had the identical staining pattern. They showed a heterogeneous PD-L1 staining among trophoblast (Fig. 1A-C). Syncytiotrophoblast had strong membrane staining, whereas most cytotrophoblasts lacked PDL1 expression. Both the villous and extravillous (placental site) intermediate trophoblasts exhibited variable staining (20%-100%), with a lower intensity (usually moderate; Fig. 1D and E). Similarly, PD-L1 staining was present in 40% to 70% of intermediate trophoblasts of the chorionic laeve in the fetal membranes with a mild-to-moderate staining intensity (Fig. 1F and G).

3.2. PD-L1 expression in HMs

All CHMs, PHMs, and IHMs showed the same PD-L1 staining as normal placentas—strong membrane staining on syncytiotrophoblast and negative staining in cytotrophoblast (Fig. 2A-F). However, exuberantly hyperplastic villous intermediate trophoblasts in IHM were diffusely and strongly positive for PD-L1 (Fig. 2G-I).

3.3. PD-L1 expression in trophoblastic tumor–like lesions

All 11 EPS cases showed membrane PD-L1 staining in 50% to 90% of trophoblasts (Fig. 3A and B). Strong staining intensity was observed in 2 cases, moderate in 7, and weak in 2. Positive PD-L1 staining was observed in 7 of 19 PSNs consisting of 2+ in 1 case and 1+ in 6. PSNs harbored relatively weak membranous staining but strong cytoplasmic staining occasionally (Fig. 3C and D). The PD-L1 expression was significantly lower in PSN (composite immunoscore; mean ± SD, 1.9 ± 1.7) compared with EPS (mean ± SD, 6.9 ± 2.7; $P < .001$).

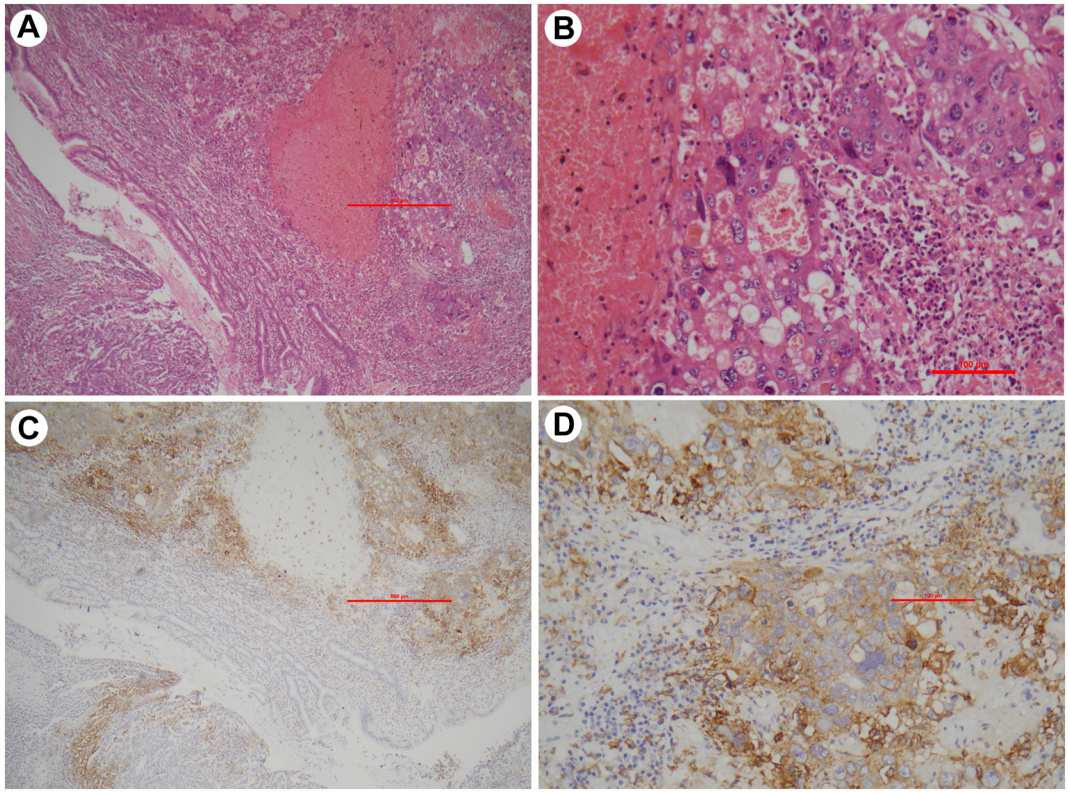

Fig. 5 PD-L1 expression in nongestational trophoblastic tumors. Gastric CCs (A and B) are characteristic of strong PD-L1 expression (C and D), whereas the poorly differentiated adenocarcinoma and normal glands show negative staining (the lower part in panel C). The peritumoral lymphocytes are PDL-1 positive (C and D).H&E: A and B. Immunohistochemistry: C and D. Original magnifications ×5 (A and C; red scale, 500 μm) and ×20 (B and D; red scale, 100 μm).

3.4. PD-L1 expression in GTNs

Details of the extent and intensity of staining in GTNs are given in Table 2. All CCs, ETTs, and PSTTs were positive for PD-L1 (Fig. 4A-I). Strong positive PD-L1 staining (composite immunoscore ≥4) was observed in nearly all GTNs. Three CCs and 2 ETTs with weak PD-L1 staining contained scant viable trophoblast. CC showed a heterogeneous staining pattern as in the placenta: diffuse and strong in syncytiotrophoblast and variable in the mononucleated trophoblast ranging from undetectable to strongly positive. ETT showed a heterogeneous PD-L1 staining pattern (10%-70%), whereas PSTT frequently demonstrated a diffuse staining pattern with a moderate-to-strong intensity. PD-L1 expression was lower in ETTs than in PSTTs and CCs ($P = .004$, Table 2). No statistical relationship could be found between PD-L1 expression and patient survival (immunoscore: survival versus death of disease, 11.1 ± 1.7 versus 10.3 ± 2.1; $P > .05$) or FIGO prognostic scores (immunoscore: low risk versus high risk, 11.3 ± 1.5 versus 10.9 ± 2.2; $P > .05$) because of strong PD-L1 expression in nearly all tumors and a small number of cases with clinical follow-up.

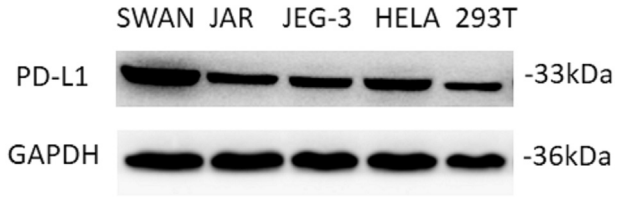

Fig. 6 PD-L1 specificity as determined by Western blot analysis.

3.5. PD-L1 expression in nongestational trophoblastic tumors

Like gestational CC, 3 germ cell–derived pure CCs, the CC elements in 13 mixed germ cell tumors, and 4 gastric/rectal CCs (Fig. 5) were heterogeneously positive for PD-L1 (Table 2). There were no statistically significant differences in PD-L1 expression between gestational and nongestational CCs ($P > .05$). The embryonic carcinoma element in 2 mixed germ cell tumors showed focal PD-L1 positivity, whereas other elements, such as yolk sac tumor, teratoma, germinoma/dysgerminoma, and adenocarcinoma, were PD-L1 negative.

3.6. PD-L1 expression in the background nontrophoblastic normal tissues

Negative expression of PD-L1 was typically observed in most background nontrophoblastic normal tissues with the exception of nerves, some tumor-infiltrating lymphocytes, and

macrophages (cytoplasmic staining). The PD-L1–negative normal cells and tissues included amnion cells, villous mesenchymal cells, stroma/decidua, myometrium/myosalpinx/muscularis proper, endometrial glands, endocervical glands, squamous epithelium, fallopian tubal mucosa, intestinal mucosa, bronchial mucosa, and alveolar epithelium.

3.7. Western blot analysis

Consistent with the immunohistochemical staining results, Western blot analysis showed that PD-L1 was consistently expressed in the trophoblast cell line, SWAN-71, and the 2 CC cell lines, JEG-3 and JAR (Fig. 6). The PD-L1 expression level seemed to be higher in SWAN-71 than in JEG-3 and JAR.

4. Discussion

The PD-1/PD-L1 axis is crucial for the regulation of immunosuppressive functions and the maintenance of immune tolerance [10]. As a maternal-fetal interface, the trophoblast in the placenta plays an essential role in the suppression of maternal immunological rejection of the fetus [15]. PD-L1 is expressed in the syncytiotrophoblast and cytotrophoblast in the human placenta [16]. In fact, it is demonstrated that the PD-1/PD-L1–negative costimulatory pathway is crucial for the fetomaternal tolerance in mouse models [15,20,21]. PD-L1–deficient female mice showed decreased allogeneic fetal survival rates compared with littermate and heterozygote controls [20].

In our study, we confirm that PD-L1 is invariably expressed in all trophoblastic lineages except cytotrophoblast. In keeping with 2 recent studies [17,18], we observed strong PD-L1 staining intensity in the syncytiotrophoblast, the terminally differentiated form of the trophoblast at the outer barrier of the villi. Nevertheless, Bolze et al [19] found strong PD-L1 expression in cytotrophoblast, which was different from ours and other studies [17,18]. The conflicting staining pattern was partially explained by the different antibodies and other technical points including antigen retrieval among studies, but we have validated the specificity of the PD-L1 antibody in our study by Western blot analysis in trophoblast cell lines. Several of our immunohistochemical findings—the diffuse PD-L1 staining with moderate intensity of the intermediate trophoblast, which frequently interact with immune cells; the negative staining for PD-L1 in cytotrophoblast, the stem cell of trophoblast [22]; and the unvarying expression pattern with gestational age and pregnancy site—suggest that PD-L1 is critical for maintaining the fetomaternal tolerance throughout the whole process of pregnancy in humans.

Our study represents the largest clinical investigation on a wide range of trophoblastic diseases to date. Our data show that PD-L1 is expressed in nearly all trophoblastic diseases except PSNs, consistent with 2 recent studies containing a small number of gestational trophoblastic diseases [17,19]. Veras et al [18] found that PD-L1 was undetectable in 5 of 30 CCs, 3 of 6 ETTs, and 6 of 14 PSTTs. Nevertheless, that study used tissue microarrays, which may cause false-negative results on the account of the heterogeneous PD-L1 staining in GTNs. The different antibodies that were used in our and previous studies may also be another explanation for the staining results. Like inhibin [23], HLA-G [24], and hydroxyl-delta-5-steroid dehydrogenase (HSD3B1) [25], we suggest that PD-L1 is a sensitive marker for trophoblastic tumors.

Nevertheless, PD-L1 is not specific for GTNs owing to its expression in many cancers [11,17]. The major technical advantage is the strong membrane staining of PD-L1 in most GTNs. Moreover, with the burgeoning of anti–PD-L1 immunotherapy, this antibody will be available in most laboratories. Therefore, PD-L1 can be included as a trophoblastic marker. However, PD-L1 does not help in the differential diagnosis of different types of trophoblastic tumors because PD-L1 is expressed in all types of GTN. The combination of other relatively lineage-specific markers, such as human placental lactogen, CD146 (Mel-CAM) [26], and p63 [27], is required to differentiate the subtypes of GTN.

Problems may sometimes arise in the differential diagnosis among GTN, poorly differentiated endometrial carcinoma, and other malignancies, especially in small biopsies. CC can occur in the context of adenocarcinoma and germ cell tumors or occur purely at extrauterine sites including the gonads and gastrointestinal tract.

Under such circumstances, the diagnosis of CC is critical because nongestational CCs have a more aggressive clinical course [6]. ETT can arise in the lower uterine segment and the cervix, metastasize to the lung, and histologically simulate squamous cell carcinoma [7,9]. Moreover, both ETT and squamous cell carcinoma are diffusely positive for p63 [27]. In addition, PSTT can morphologically mimic uterine leiomyosarcoma, particularly the epithelioid subtype [8]. Previous studies have indicated that many cancers, such as squamous cell carcinomas (uterine cervix and lung), endometrial carcinomas, colorectal carcinomas, and leiomyosarcomas, have variable PD-L1 staining [11,17,28], with most cases showing focal staining.

We here observed that PD-L1 expression was present in primary gastrointestinal CCs rather than in the concurrent adenocarcinomas for the first time. Focal PD-L1 was found in 2 embryonic carcinomatous components of 13 mixed germ cell tumors in keeping with a previous report [29]. In contrast, most GTNs and nongestational CCs had a diffuse and strongly positive PD-L1 staining. Therefore, PD-L1, together with a panel of other antibodies including cytokeratins, desmin, smooth muscle actin, p16, GATA3 [30], SALL4 [31], OCT4 [32], and other trophoblast-specific markers [23-25], is helpful in the recognition of trophoblastic tumors among other cancers.

PD-L1 immunostaining is paramount for future anti–PD-L1 immunotherapies in trophoblastic tumors. Immunotherapy targeting the PD-1/PD-L1 axis has shown substantial efficacy and promise for many solid tumors at advanced stage and wide metastasis, such as lung, colorectal, and gastric cancers

[13,33]. Clinical trials on the anti–PD1/PD-L1 axis have indicated a tendency for better therapeutic efficacy with higher PD-L1 immunostaining in tumor samples. Most previous studies proposed a 5% cutoff value for positive PD-L1 expression in solid tumors [11,14,28]. Most positive cases showed marginal staining on the interface between the tumor and stroma, and a diffuse strong staining pattern was uncommon in solid tumors.

In contrast, our data clearly demonstrated a strong PD-L1 expression in most trophoblastic tumors. Therefore, we provide a strong rationale for future anti–PD-L1 therapy in trophoblastic tumors. Approximately 10% GTN patients, particularly patients with PSTT and ETT, are insensitive to chemotherapy and may have fatal clinical courses [2,3]. Our pooled analysis indicated that ETT patients had a high rate of metastasis and mortality, and did not benefit from chemotherapy [34]. Compared with ETT, most PSTT patients showed a relatively favorable prognosis. Young women with PSTT, who had a strong desire for fertility preservation, could be treated with local excision but showed a high tumor recurrence and potential risks for tumor spread [35].

Nongestational CCs, particularly somatic cell–derived CCs, showed a much worse clinical outcome than did gestational CCs. A pooled analysis of 53 gastric primary CCs showed an extremely poor prognosis even with a combination of curative surgery and chemotherapy: 47 patients died within 1 year and only 1 was alive for more than 1 year [6]. Therefore, our findings of diffuse PD-L1 expression in PSTT, ETT, and somatic CC generate a strong rational for future immunotherapy, which may lower tumor relapse in PSTT women with fertility-sparing surgery, or eventually improve the prognosis in highly aggressive ETT and somatic CC.

In summary, PD-L1 is widely expressed in the normal placenta, molar pregnancies, and gestational and nongestational trophoblastic tumors. Being a sensitive but nonspecific marker, PD-L1 can aid in the discrimination of trophoblastic tumors from other cancers. More importantly, frequent strong PD-L1 expression suggests that immune checkpoint blockade could be a promising approach in treating trophoblastic tumors that merits clinical trials for further investigation.

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