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Characterization of immune regulatory molecules B7-H4 and PD-L1 in low and high grade endometrial tumors

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HIGHLIGHTS

- Endometrial tumors with microsatellite instability and high grade have elevated PD-L1 protein levels.
- Protein levels of B7-H4 are prevalent and independent of microsatellite instability, grade and histology.
- Combination immunotherapies directed against checkpoint proteins may hold promise for endometrial carcinoma.

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ABSTRACT

Background. The objective of this investigation was to characterize the expression landscape of immune regulatory molecules programmed death-ligand-1 (PD-L1, B7-H1) and B7-H4 in a cohort of endometrial tumors across the spectrum of grade and histology.

Materials and methods. With institutional review board approval, 70 endometrial tumors from patients with known clinical outcomes were identified representing a spectrum of grade and histology. Immunohistochemistry (IHC) was performed for PD-L1 and B7-H4 and scored. Microsatellite instability (MSI) status was assessed for endometrioid tumors using the institutional IHC assay for expression of the mismatch repair (MMR) genes, MLH1, MSH2, MSH6 and PMS2. RNA sequencing data from the Cancer Genome Atlas was queried for expression levels of *CD274* (PD-L1 protein) and *VTCN1* (B7-H4) across molecular subtypes of endometrial carcinoma and were correlated with a T cell infiltration index.

Results. We identified 40 low grade endometrioid tumors and a cohort of 30 high grade tumors. PD-L1 expression was observed in both high and low grade endometrial tumors (56% vs 35%, $p = 0.07$). In the low grade tumors, PD-L1 expression was associated with MSI status ($p < 0.01$). The high grade cohort had similar rates of PD-L1 expression compared to low grade MSI tumor (56% and 62% respectively), and both were distinct from low grade MSS tumors (22%, $p < 0.05$). High (3+) B7-H4 positive cells were observed in both high and low grade carcinomas (33% and 31% respectively). RNA profiling data from confirmed highest *CD274* expression in POLE and MSI tumors that was linearly correlated with T cell infiltration, while *VTCN1* expression appeared consistent across molecular subtypes.

Conclusions. While PD-L1 expression correlated with MSI and high grade tumors, B7-H4 expression was independent of grade, histology and immune cell infiltration. The development and testing of multi-agent therapeutics targeting PD-L1 and B7-H4 may be a novel strategy for endometrial tumors.

1. Introduction

Endometrial cancer (EnCa) is the most common gynecologic malignancy accounting for over 60,000 newly diagnosed cases and over 8000 deaths in the United States in 2016 [1]. Marked differences in clinical

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behavior have been observed in patients with EnCa depending on the histologic subtype, the tumor grade and the extent of cancer spread. Investigators have suggested a classification system that separates endometrial tumors into type I and type II subsets to account for the striking divide in risk factors, clinical behavior and approach to therapy [2]. While type II cancers account for only 15–25% of all EnCa, patients with these tumors account for 75% of the mortality observed highlighting the need for novel therapies beyond conventional surgery, radiation and cytotoxic chemotherapy for this subset of tumors [3].

Recently, the clinical type I/II distinction has been reframed into molecular categories by TCGA with distinctive molecular signatures that define prognosis [4]. The categories described are 1) POLE associated with mutation in the DNA polymerase gene E marked by ultramutation, 2) microsatellite instability (MSI) associated with mutations in the DNA repair machinery, marked by hypermutation, 3) microsatellites stable (MSS) marked by endometrioid histology and 4) DNA copy number high marked by serous-like histology. Importantly, copy number high, predominantly serous like tumors portended the most guarded prognosis, with the ultramutated POLE group being associated with the best prognosis [4].

Endometrial cancers with POLE mutation or MSI tumors can harbor 10 to 100 times more mutations as compared to the MSS tumors [4]. Those tumors with high mutational burden can harbor potent antigens and therefore are subject to host immune surveillance which is possibly associated with improved prognosis observed in these tumors [5–7]. Given this hypothesis, when these subsets of tumors do recur, there is increased reliance on immune cloaking mechanisms for survival and metastasis [8]. These tumors may contain prominent immune cell infiltrates and express high levels of immune checkpoint molecules that dampen the host immune response resulting in tumor growth [9].

Recent clinical studies have demonstrated durable clinical benefit with antibody therapies that target immune checkpoints [9–11]. Programmed death 1 (PD-1, B7-H1) is a member of B7 receptors that modulate T cell response [12]. Expression of the checkpoint molecule PD-1 on immune lymphocytes has been shown to limit T cell response through the interaction with either or both of its ligands, PD-L1 and PD-L2 [12]. Indeed, as a mechanism of immune evasion, tumors have been shown to upregulate PD-L1 expression on tumor cells further suppressing T cell activity promoting immune evasion and enhancing tumor survival [13]. Antibody therapies that target PD-1 (Pembrolizumab, Nivolumab, etc.) or PD-L1 (Atezolizumab, Avelumab, etc.), and disrupt the PD-1/PD-L1 interaction, have shown marked anti-tumor efficacy in numerous indications including, melanoma, non–small-cell lung cancer, renal-cell carcinoma, and Hodgkin's lymphoma [10]. These immune checkpoint blockade therapies neutralize the inhibitory signals generated the checkpoint molecules resulting in induction, activation and expansion of T cells that eliminate the tumor [13].

Recent clinical studies suggest that MSI status predicted clinical benefit to anti-PD-1 therapy with Pembrolizumab [14]. In this study, patients with tumors harboring MSS had an 11% clinical benefit rate (CR + PR + SD) compared to a 70% clinical benefit rate in patients with tumors with MSI [14]. The MSI tumors presented with an over ten-fold higher mutational burden and significantly elevated levels of PD-L1 expression at the invasive front of the tumors when compared to the MSS tumors [14]. Numerous other correlative studies in additional indications have echoed these results and found significantly higher response rates in those tumors expressing PD-L1 [9,15].

Similar to PD-L1, another member of the B7 family, B7-H4 (a.k.a. VTCN1, B7h.5, B7S1, B7x) suppresses effector function of T cells through interaction with an unknown receptor expressed on T cells. Although the exact mechanism of T cell inhibition is not known, based on published reports B7-H4 is thought to mediate tumor immune evasion by inhibiting activation of T and NK cells [16,17]. B7-H4 has been reported to be frequently overexpressed by tumor cells in a variety of solid tumors including ovarian, lung, breast, pancreatic and renal cell [16–21]. Expression of B7-H4 has also been reported to inversely correlate with T cell infiltration [22].

EnCa is a tumor marked by diverse grade and histology with significant subsets harboring high mutational burdens in concert with MSI [4]. We hypothesize that this intrinsic nature predisposes EnCa to being responsive to this new class of immune sensitization therapy and that understanding the baseline expression profiles of key immune checkpoint proteins in endometrial tumors will be crucial for identifying those patients most likely to respond this new class of immunotherapies. We analyzed the TCGA data for expression of PD-L1 and B7-H4 and found that immune infiltration signatures as well as PD-L1 and B7-H4 expression were manifest most in MSI and POLE tumors when compared to MSS tumors. We then conducted a molecular analysis of 70 endometrial tumors from the range of histologic subtypes and found that high grade tumors and those low grade endometrioid tumors that harbor MSI express higher PD-L1 and B7-H4 protein levels when compared to low grade endometrioid MSS tumors. Collectively, these data suggest that a range of endometrial tumors harbor signatures that may be amenable to immune modulation through interactions with PD-1 and B7-H4.

2. Materials and methods

2.1. Patients and samples

Under an institutionally approved research plan and agreement, we identified 70 endometrial carcinomas across a spectrum of grade and histology of patients diagnosed between 2010 and 2013 with complete clinical follow up and available tissue for analysis. Clinical factors were extracted from patient records, including age, grade, stage, treatment, recurrence and survival. All molecular analyses were carried out on formalin-fixed and paraffin-embedded (FFPE) diagnostic specimens. Hematoxylin and eosin-stained slides were marked for tumor location by a gynecologic oncology pathologist.

2.2. Immunohistochemistry

Paraffin embedded EnCa FFPE tissue sections of 5 μm thickness were subjected to immunohistochemistry (IHC) for PD-L1 and B7-H4 using clones D1M8I (B7-H4), E1L3N (PD-L1) (Cell Signaling, Waltham MA), following protocols established for staining on the Leica BONDRX automated platform. Scoring for both markers was performed independently by two operators according to guidelines. Briefly, B7-H4 expression was scored on an intensity scale of 0 to 3+ and reported as an H-score calculated as (intensity score) ∗ (% cells for each staining

Table 1
Cohort Characteristics.

Age (range)		64.9 (39.6–88.7)
Stage	1	50 (71%)
	2	3 (4%)
	3	9 (13%)
	4	8 (11%)
Grade	1	37 (53%)
	2	4 (6%)
	3	29 (41%)
Histology	High grade endometrioid	10 (14%)
	Low grade endometrioid	40 (58%)
	Carcinosarcoma	10 (14%)
	Uterine serous carcinoma	10 (14%)
MSI Status	Endometrioid MSI-high	13 (33%)
	Endometrioid MSS	27 (67%)
	Not assessed	30
Recurrence (%)	Total	15 (21%)
	High grade endometrioid	1 (10%)
	Carcinosarcoma	5 (50%)
	Low grade endometrioid MSI-high	1 (8%)
	Low grade endometrioid MSS	3 (13%)
	Uterine serous carcinoma	5 (50%)