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B7-H4 Expression in Ovarian Serous Carcinoma: A Study of 306 Cases

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Abstract

The B7 family of immune costimulatory ligands is a group of cell surface proteins that bind to the surface receptors of lymphocytes to fine-tune immune responses. The aberrant expression of these proteins plays a key role in tumor immune evasion. Immunotherapy targeting certain B7 family members, including programmed death ligand 1, has proven quite effective in suppressing tumor growth. However, why such therapy works in only a subgroup of tumors is unclear. We hypothesized that other B7 family members, either alone or in concert with programmed death ligand 1, play a crucial role in tumor pathogenesis and progression. We therefore examined the expression of a newly discovered B7 family member, B7-H4, in 306 cases of ovarian serous carcinoma by immunohistochemistry. We found that 91% (267/293) of the high-grade ovarian serous carcinomas and 69% (9/13) of the low-grade ovarian serous carcinomas expressed B7-H4. The difference between B7-H4 expression in high-grade and low-grade ovarian serous carcinoma was statistically significant ($P = 0.002$). Moreover, B7-H4 protein expression in high-grade serous carcinoma was associated with tumor stage ($P < 0.01$) but not overall survival or disease-free

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survival. In conclusion, B7-H4 is frequently expressed in ovarian serous carcinomas, especially high-grade serous carcinomas, and may represent a novel immunotherapeutic target in this cancer.

Keywords

Ovarian cancer; fallopian tube; B7-H4; immunohistochemistry; immunotherapy

1. Introduction

The B7 family of immune costimulatory ligands is a group of cell surface proteins that bind to the surface receptors of lymphocytes to fine-tune immune responses. The aberrant expression of these proteins plays a key role in tumor immune evasion [1-4]. Immunotherapy targeting certain B7 family members, including programmed death ligand 1 (PD-L1), has proven quite effective in suppressing tumor growth. However, why such therapy works in only a subgroup of tumors is unclear. We hypothesized that other B7 family members, either alone or in concert with programmed death ligand 1, play a crucial role in tumor pathogenesis and progression.

One recently discovered member of the B7 family is B7-H4. The human *B7-H4* gene is located on chromosome 1, and a possible *B7-H4* pseudogene is located on chromosome 20. The *B7-H4* gene has six exons and five introns, and the first two exons encode a signal peptide. Alternative splicing of the *B7-H4* gene produces two different transcripts [5]. Although many normal human tissues (e.g., lung, liver, kidney, ovary, testis, placenta) express B7-H4 messenger RNA (mRNA), normal tissues have no or little B7-H4 protein expression, which suggests that B7-H4 expression is regulated post-transcriptionally [5]. B7-H4 has been shown to inhibit T-lymphocyte proliferation and cytokine production and thus may serve as a means by which some ovarian serous carcinomas circumvent anti–PD-L1 immunotherapy [5]. Although some small studies have reported B7-H4 protein expression in ovarian cancers [5-9], the patterns, rates, and levels of B7-H4 expression in ovarian serous carcinoma by histological grade or disease stage have not been assessed definitively in a large-scale study.

In the present study, to determine the potential of B7-H4 as an immunotherapeutic target, we assessed its expression in a large number of ovarian serous carcinoma samples. Our study's findings may provide the basis for expanding the scope of immunotherapeutic drugs used against this disease.

2. Materials and Methods

2.1. Patients and samples

With approval from the Institutional Review Board, we identified 306 patients who underwent surgery for ovarian serous carcinoma at our institution between 1990 and 2009. The patients' relevant clinical data, including demographic information, pathologic diagnosis, laboratory findings, radiologic findings, and follow-up information, were obtained from electronic medical records. The diagnosis of ovarian serous carcinoma was based on 2014 World Health Organization criteria [10]. Ovarian serous carcinomas were graded using

a 2-tier system (low-grade versus high-grade) [11] and staged using the International Federation of Gynecology and Obstetrics (FIGO) system [12]. In addition, we assessed the expression of B7-H4 in normal ovary (n = 24) and fallopian tube (n = 20). Tissue microarrays were constructed as described previously [13-17].

2.2. Immunohistochemical staining for B7-H4

Immunohistochemical staining for B7-H4 was performed with an anti–B7-H4 rabbit monoclonal antibody (D1M8I, 1:200; Cell Signaling, Danvers, MA) as per the manufacturer's recommendations. The patients' formalin-fixed, paraffin-embedded tissue specimens (3-μm-thick sections) were deparaffinized, exposed to Peroxidazed 1 (PX968; Biocare Medical, Concord, CA, USA) for 5 minutes to decrease background staining, and then immersed in a universal decloaker (UD1000M; Biocare Medical) and placed in a pressure cooker at 121°C for 5 minutes. Next, the sections were blocked using blocking reagent (BS966M; Biocare Medical) for 30 minutes, incubated with primary antibody overnight at 4°C, incubated with the biotinylated secondary antibody (GU600H; Biocare Medical) for 10 minutes, and incubated with Streptavidin HRP Label (HP604; Biocare Medical) for 10 minutes. Finally, the sections were stained with 3,3′-diaminobenzidine chromogen (DB801L; Biocare Medical) and then counterstained with hematoxylin.

The pattern of B7-H4 immunohistochemical staining was recorded using a 4-score grading system (Figure 1): Score 0, no staining/negative; Score 1, apical pattern; Score 2, mixed apical and circumferential membranous staining with circumferential membranous staining in <10% of tumor cells; and Score 3, circumferential membranous staining in ≥10% of tumor cells.

2.3. Statistical analysis

Summary statistics including mean, median, and range were provided for the continuous variable age. Categorical variables such as histologic types and FIGO stage were summarized in count and frequency. The Fisher's exact test was used to compare the expression of B7-H4 between high-grade and low-grade ovarian serous carcinomas. To assess the effects of the B7-H4 immunohistochemical score on the tumor stage, we performed logistic regression analysis, in which patients with the stage I or II disease were combined in an early-stage disease group and patients with stage III or IV disease were combined in an advanced-stage disease group. P-values < 0.05 were considered statistically significant.

Disease-free survival (DFS) was measured from the date of original diagnosis to the date of tumor relapse (i.e., the appearance of recurrent lesions or a doubling of serum CA125 levels from the upper limit of normal) or last follow-up. Overall survival (OS) was measured from the date of original diagnosis to the date of death or last follow-up [13, 17]. OS and DFS curves were estimated using the Kaplan-Meier method and compared using log-rank tests. Cox proportional hazards regression models were used to assess the effect of the B7-H4 immunohistochemical score on OS and DFS. All statistical analyses were performed with SAS (version 9.3, SAS Institute, Cary, NC).

3. Results

3.1 B7-H4 expression in normal ovary and fallopian tube

B7-H4 was not expressed in normal ovary (n = 24; Figure 1a). However, some epithelial cells in normal fallopian tube showed an apical pattern of B7-H4 expression (n = 20; Figure 1b).

3.2 B7-H4 expression in high-grade and low-grade ovarian serous carcinoma

The patients' clinical characteristics are summarized in Table 1. The expression of B7-H4 in high-grade and low-grade ovarian serous carcinomas is summarized in Table 2. We found that 91% (267/293) of the high-grade ovarian serous carcinomas and 69% (9/13) of the low-grade ovarian serous carcinomas expressed B7-H4. The difference between B7-H4 expression in high-grade and low-grade ovarian serous carcinoma was statistically significant ($P = 0.002$).

3.3 B7-H4 expression in high-grade ovarian serous carcinoma is associated with tumor stage but not OS or DFS

The results of the logistic regression models for high-grade serous carcinoma are given in Table 3. The association between the B7-H4 immunohistochemical score and tumor stage was statistically significant in high-grade serous carcinoma ($P < 0.01$). Patients with a higher B7-H4 immunohistochemical score had a higher risk of having an advanced tumor stage.

The Kaplan-Meier estimates of the OS and DFS rates of patients with high-grade or low-grade serous carcinoma are shown in Figure 2. The OS and DFS rates of patients with low-grade serous carcinoma were significantly longer than those of patients with high-grade serous carcinoma ($P = 0.014$ and 0.041, respectively). We found no significant association between B7-H4 immunohistochemical score and OS or DFS (Table 4).

4. Discussion

We found that the B7-H4 protein was frequently expressed in ovarian serous carcinoma, especially in high-grade serous carcinoma. The tumor specimens had different B7-H4 staining patterns, including apical, circumferential membranous, and mixed apical and circumferential membranous patterns. The immunohistochemistry score of B7-H4 protein expression in high-grade serous carcinoma was associated with tumor stage. These findings suggest that B7-H4 is a promising immunotherapeutic target for ovarian serous carcinoma.

Our results are largely in keeping with those of earlier studies with smaller sample sizes [5-9]. Choi and colleagues [5], using the monoclonal antibodies hH4.3 and hH4.2 and defining positive staining as B7-H4 positivity in >10% of tumor cells regardless of the immunostaining pattern, found that 85% (22/26) of ovarian tumors expressed B7-H4 [5]. Tringeler and colleagues [7], using the monoclonal antibody A57.1 and a three-tier system identifying negative, apical, and circumferential membranous and strong cytoplasmic staining patterns, found that 100% (32/32) of ovarian serous carcinomas, 78% (18/23) of serous borderline tumors, and 77% (20/26) of serous cystadenomas expressed B7-H4. Moreover, the authors found that a circumferential membranous and strong cytoplasmic

pattern of B7-H4 staining occurred mainly in invasive ovarian carcinomas. Dangaj and colleagues [8], using flow cytometry, demonstrated cell surface expression of B7-H4 in 100% (15/15) of ovarian cancer samples. In our study, since a subset of the cases showed a mixed apical and circumferential membranous pattern of B7-H4 staining, we used a four-tier system to interpret immunohistochemistry results.

In addition, earlier studies showed that whereas B7-H4 messenger RNA is expressed in many normal tissues, B7-H4 protein is expressed in only antigen-presenting cells and cancer cells, likely because the protein is regulated post-transcriptionally [5]. Therefore, reverse-transcriptase polymerase chain reaction, mRNA sequencing, and RNA in-situ hybridization are not useful in studying B7-H4 expression. In the present study, B7-H4 was not expressed in normal ovary. Although B7-H4 was expressed in some epithelial cells in normal fallopian tube, it demonstrated an apical (incomplete membranous) pattern of expression instead of the circumferential membranous pattern seen in the majority of high-grade ovarian serous carcinomas. The mechanism of this phenomenon is unclear and needs be studied further. Previous studies have used the enzyme-linked immunosorbent assay to detect the B7-H4 protein in serum and ovarian cancer tissue lysates [18-20]. Serum B7-H4 level is a potential diagnostic marker of ovarian cancer. Our results also suggest that for high-grade ovarian serous carcinoma, B7-H4 expression is associated with tumor stage but not DFS or OS. However, Kryczek and colleagues [9] reported that B7-H4 expression in tumor-associated macrophages was associated with worse prognosis in ovarian cancer patients.

Currently, immunotherapeutic drugs targeting B7-H4 is under development. Dangaj and colleagues [8, 21, 22] found that the intraperitoneal injection of anti–B7-H4 single-chain variable fragments slowed the growth of OCVAR-5–derived ovarian cancer xenografts. In the future, anti–B7-H4 therapy may be tested in patient-derived xenograft models and/or clinical trials. Our results, together with those of other authors [23], suggest that B7-H4 is a promising immunotherapeutic target in ovarian cancer.

In conclusion, our large cohort study showed that ovarian serous carcinomas frequently express B7-H4, which raises the possibility that the immunomodulatory molecule is a promising immunotherapeutic target in ovarian cancers. Moreover, the assessment of other B7 family members (i.e., B7.1, B7.2, inducible costimulator ligand, B7-H3, B7-H5, B7-H6, B7-H7) in ovarian and other types of cancers may also expand our knowledge of tumor immune escape and provide new immunotherapeutic targets.

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Research highlights

- B7-H4 is a member of the B7 family of immune costimulatory ligands.
- B7-H4 protein is frequently expressed in ovarian serous carcinoma, especially in high-grade serous carcinoma.
- B7-H4 may represent a novel immunotherapeutic target in ovarian cancer.

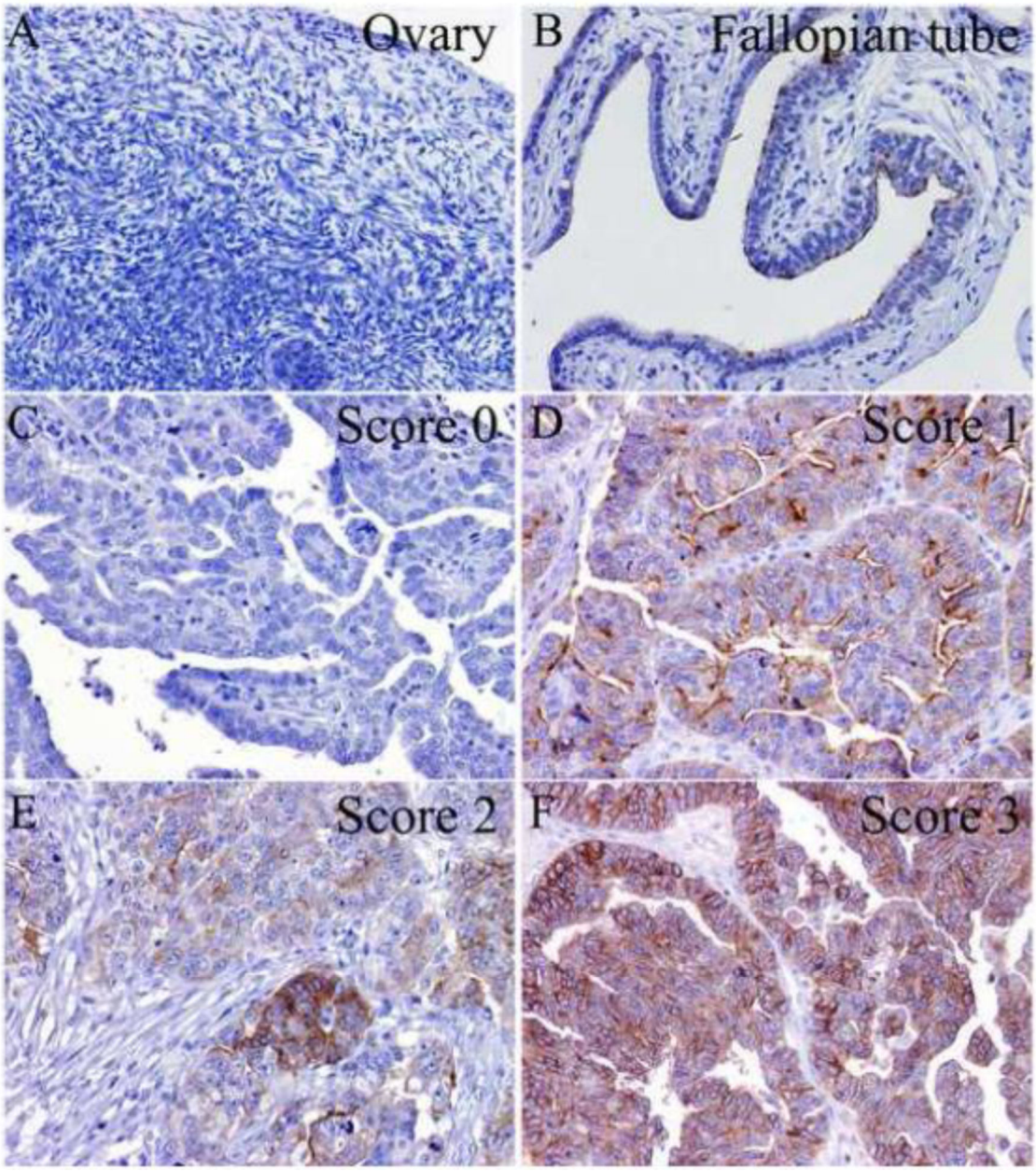

Fig. 1.

B7-H4 protein expression in normal ovary (A), fallopian tube (B), and high-grade ovarian serous carcinoma (C-F). B7-H4 immunohistochemical staining in ovarian serous carcinoma was semi-quantified using a four-score grading system: Score 0 (C), negative; Score 1 (D), apical pattern; Score 2 (E), mixed apical and circumferential membranous staining with circumferential membranous staining in <10% of tumor cells; Score 3 (F), circumferential membranous staining in ≥10% of tumor cells (immunohistochemical stain; original magnification ×200).

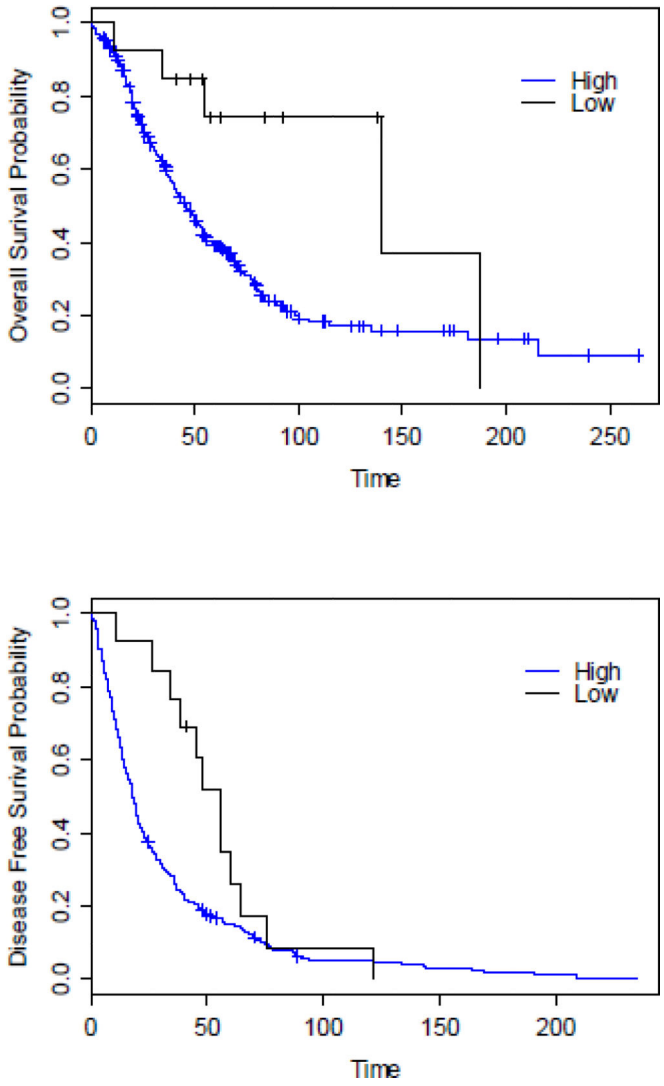

Fig. 2.

Kaplan-Meier estimates of the overall survival (OS) and disease-free survival (DFS) rates of patients with high-grade ovarian serous carcinoma versus those with low-grade ovarian serous carcinoma ($P = 0.017$ for OS and $P = 0.041$ for DFS).

Table 1

Clinicopathologic data of 306 patients with ovarian serous carcinoma.

Histologic types	
Low-grade serous carcinoma	13
High-grade serous carcinoma	293
Age at diagnosis (years)	
Mean	60
Median	61
Range	22-87
FIGO Stage	
I	6
II	9
III	211
IV	68
Unknown	12

Table 2

B7-H4 immunohistochemical scores in high-grade versus low-grade ovarian serous carcinoma.

	B7-H4 Immunohistochemical Score				
	0	**1**	**2**	**3**	**Total**
High-grade serous carcinoma					
Stage I&II	1	5	4	4	14
Stage III	18	49	43	90	200
Stage IV	7	6	19	36	68
Stage	0	3	4	4	11
Unknown					
Total (%)	26 (8.9%)	63 (21.5%)	70 (23.9%)	134 (45.7%)	293 (100%)
Low-grade serous carcinoma					
Stage I&II	0	1	0	0	1
Stage III	4	5	1	1	11
Stage IV	0	0	0	0	0
Stage	0	0	1	0	1
Unknown					
Total (%)	4 (30.8%)	6 (46.2%)	2 (15.4%)	1 (7.7%)	13 (100%)

Table 3

Ordered logistic regression for high-grade ovarian serous carcinoma with tumor stage as dependent variable.

Characteristic		Coefficient (95% CI)	P-value
B7-H4 IHC score			**<0.01**
	1	−1.04 (−2.09,0.01)	0.05
	2	0.01 (−0.97,0.99)	0.99
	3	0.06 (−0.86,0.97)	0.90
	0		
Tumor stage (Intercept)			
	I&II	−3.25 (−4.23,−2.26)	<0.01
	III&IV	1.00 (0.17,1.84)	0.02

Table 4

Cox regression models for overall survival and disease-free survival for patients with high-grade ovarian serous carcinoma.

	Characteristic		HR (95% CI)	Cox Model P-value
Overall survival	**B7-H4 IHC score**			**0.816**
		1	0.84 (0.48,1.48)	0.551
		2	0.78 (0.45,1.37)	0.392
		3	0.79 (0.47,1.32)	0.365
		0		
Disease-free survival	**B7-H4 IHC score**			**0.657**
		1	0.77 (0.47,1.25)	0.282
		2	0.76 (0.47,1.24)	0.268
		3	0.84 (0.54,1.32)	0.457
		0		

HR, Hazard Ratio; CI, Confidence Interval.