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B7-H4 expression in ovarian serous carcinoma: a study of 306 cases☆,☆☆

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Summary 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 ($P = .002$). Moreover, B7-H4 protein expression in high-grade serous carcinoma was associated with tumor stage ($P < .01$) but not overall survival or disease-free 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.

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),

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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 (eg, 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) 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 (Fig. 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. $P < .05$ was considered statistically significant.

Disease-free survival (DFS) was measured from the date of original diagnosis to the date of tumor relapse (ie, 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; Fig. 1A). However, some epithelial cells in normal fallopian tube showed an apical pattern of B7-H4 expression (n = 20; Fig. 1B).