Annals of Medical Research

DOI: 10.5455/annalsmedres.2019.09.524 2020;27(1):283-91

Original Article

Prognostic significance of B7H4 expression in patients with optimally or maximally cytoreduced ovarian clear cell carcinoma

Zeliha Firat Cuylan[1], Koray Aslan[1], Kamil Hakan Muftuoglu[2], Murat Oz[1], Mehmet Mutlu Meydanli[1]

[1]Zekai Tahir Burak Womens Health Training and Research Hospital, Faculty of Medicine, University of Health Sciences, Department of Gynecologic Oncology, Ankara, Turkey
[2]Zekai Tahir Burak Womens Health Training and Research Hospital, Faculty of Medicine, University of Health Sciences, Department of Pathology, Ankara, Turkey

Abstract

Aim: Ovarian clear cell carcinoma is known as an aggressive subtype of malignant ovarian neoplasm due to being relatively resistant to platinum based chemotherapy. Therefore, more effective treatment modalities in ovarian clear cell carcinoma are needed. To investigate the association among expression levels of B7H4 with clinicopathological characteristics and also, survival outcomes of patients with ovarian clear cell carcinoma.

Material and Methods: Formalin-fixed, paraffin-embedded tissue blocks from 31 patients with histologically proven ovarian clear cell carcinoma were eligible for the study. Tissue blocks were immunohistochemically stained with B7H4 antibody and scored for expression levels of B7H4. The association between expression levels of B7H4 and the clinicopathological characteristics and survival outcomes of patients were evaluated.

Results: Maximal and optimal cytoreductive surgery rates were 45.2% and 54.8%, respectively. The 5-year disease-free survival and overall survival rates were 55.6% and 56.6% for the entire cohort, respectively. All tumor specimens had positive immunohistochemical staining with B7H4 antibody to some extent. Of the 31 patients, 14 (45.2%) showed high expression and the remaining had low expression with B7H4. There was no significant difference between expression levels of B7H4 and clinicopathological characteristics of patients. The 5-year disease-free survival rate was 52.9% in the low expression group and 61.2% in the high expression group. The 5-year overall survival rates were comparable between the low expression group and the high expression group (47.2% and 67.5%, p=0.35).

Conclusion: IB7H4 expression is a biomarker for ovarian clear cell carcinoma but it is unrelated with clinicopathologic characteristics and survival outcomes of patients.

Key words: Ovarian clear cell carcinoma; B7H4; immunotherapy; cytoreductive surgery

INTRODUCTION

B7-H4 (B7x, B7S1 or VTCNI) is type I transmembrane protein which a member of the B7 family. It consists of a transmembrane domain, one pair of V, a signal peptide region, C immunoglobulin extracellular domains and a cytoplasmic domain (1). The B7 protein family has an important role in the initiation, development and recurrence of cancer with its function in the immune system (2). The B7 protein family regulates T-cell response either inhibitory or stimulatory way, which depends on the type of B7 receptor and ligand located on the surface of the target cell (3). B7H4 is responsible for inhibition of T cell proliferation, T-cell cycle arrest, induction of T-cell apoptosis, increased production and proliferation of regulatory T cells, decreased function of antigen presenting cells (APCs) and T-cell mediated cytokine production and cytotoxicity (4-6).

B7H4 mRNA is distributed in peripheral tissues to a great extent and expressed in APCs (7,8). However, expression of B7H4 protein appears to be restricted in most of the human tissues, other than epithelial cells of the lung, the pancreas and the kidney (1). Studies have found that B7H4

Received: 19.09.2019 **Accepted:** 09.12.2019 **Available online:** 19.02.2020
Corresponding Author: Zeliha Firat Cuylan, Zekai Tahir Burak Womens Health Training and Research Hospital, Faculty of Medicine, University of Health Sciences, Department of Gynecologic Oncology, Ankara, Turkey **E-mail:** zelihafiratcuylan@gmail.com

is expressed in non-gynecologic and gynecologic cancers including endometrial, breast, lung, ovarian, cervical and renal cell cancers (4,6,9-12). It was demonstrated that the expression level of B7H4 in ovarian cancer was related with the prognosis and survival outcomes of patients in recent studies (10,13,14). Therefore, B7H4 protein can be used as a target molecule for immunotherapy in the treatment of ovarian cancer patients.

When compared to other histological subtypes of epithelial ovarian carcinomas (EOC), ovarian clear cell carcinoma (CCC) manifests a discriminate clinical behavior. Ovarian CCC, being relatively resistant to platinum based chemotherapy, is considered as an aggressive subtype of epithelial ovarian cancer, which results in poorer oncologic outcomes than ovarian high grade serous carcinoma (HGSC) (15). Therefore, more effective treatment modalities in ovarian CCC are needed. The aim of this study is to evaluate the association between expression levels of B7H4 with clinicopathological characteristics and survival outcomes of patients who have ovarian CCC.

MATERIAL and METHODS

Patients and Samples

Paraffin blocks were obtained from thirty-one patients with histologically proven ovarian CCC at our institution between December 2007 and December 2016 following the Local Institutional Review Board's approval. We got an informed consent from each patient in order to conduct the surgical procedure and they allowed us to use their medical information for research purposes at the time of admission. We obtained the clinical data of the patients, which include demographic information, pathologic diagnosis and the information regarding follow-up through electronic medical records. We performed the histologic classification according to the World Health Organization classification (16). The 2014 International Federation of Gynecology and Obstetrics (FIGO) staging system was used for staging of tumors (17). Surgical and pathological assessment was employed retrospectively to determine the disease stage in the patients who had undergone surgery before 2014.

In this study, we included women who had been subjected to primary surgical treatment which consists of total hysterectomy plus bilateral salpingo-oophorectomy, with bilateral pelvic and para-aortic lymphadenectomy and other surgical procedures with the diagnosis of ovarian CCC. Cytoreductive surgery (CRS) consisted of peritonectomy and organ resection along with staging surgery. Optimal CRS was defined as residual tumor which ranges from 0.1 to 1 cm in diameter, maximal CRS was defined as no macroscopic residual tumor (R0), and suboptimal CRS was defined as residual tumor which measures >1 cm in diameter. Gynecologic oncologists performed all the operations in order to achieve maximal cytoreduction. Women with no lymphadenectomy and histological diagnosis other than ovarian CCC were excluded. We also excluded patients who had residual tumor larger than 1 cm, women with synchronous malignancies, women to whom neoadjuvant chemotherapy is administered and those with incomplete medical records.

The multidisciplinary tumor board gave the decision to carry out adjuvant therapy. We followed-up the patients four times each year during the first 2 years, two times each year over 5 years, and annually thereafter. At the time of the last follow-up, the survival status of the patients was recorded. A social security death index search was employed to confirm each study subject with a recorded death. The description of disease-free survival (DFS) is the time beginning from the surgery to first event (recurrence or death), whichever occurs first. The time period from initial surgery to the last contact or the date of death corresponds to overall survival (OS). At their last known follow-up, the patients who survived were censored.

Immunohistochemical (IHC) staining

We placed formalin-fixed, paraffin-embedded tissue blocks on charged glass slides after sectioning them to 5 micrometers. The patients' formalin fixed, paraffin embedded tissue specimens were deparaffinized and 3.0% H2O2 was applied (Thermo Scientific) for 30 minutes to block endogenous peroxidase activity. Antigen retrieval was carried out in a citrate buffer (Thermo Scientific, Heat Induced Epitope Retrieval) at 120 °C with microwave for 15 minutes. Staining Applied with manual method by employing an indirect avidin-biotin immunoperoxidase (Thermo Scientific). The sections were incubated at 25°C for 75 minutes with B7H4 (AbCam® Anti-B7H4 antibody [MIH43], 100 µg ab110221antibody).

IHC analysis

We used a scoring system for IHC staining quantification which was described by Xu M et al. (9) previously. The corresponding microscopic images of the stained sections were obtained under a microscope at x400 magnification. Five regions of interest (ROI) were chosen at random for each section of ovarian CCC and the sections were examined by magnifying 200 times for the IHC analysis. We manually counted the number of tumor cells whose cytoplasm/cytomembrane had positive staining, and the number of total cells which had been presented in each ROI. We calculated the percentage of positive counts and reported the mean values. IHC scoring system was employed for the semi-quantitative assessment and it was described as the following: 0, no positive cells (0%); 1, 1-10% positive cells; 2, 11-50%; 3, 51-80%; and 4, 81-100%. Furthermore, the strength of staining for positive cell was evaluated and scored as the following: 0, negative; 1, weakly positive (Figure 1); 2, moderately positive (Figure 2); and 3-4, strong positive (Figure 3) staining. The IHC score for tissue specimens was defined as the multiplication of the above two parts: (-), 0 point; +, 1-4 points; ++, 5-8 points; and +++, 9-12 points. In this study, we defined the scores ≤4 points as weak or low expression and scores >4 points signified high expression.

Statistical analysis

For the survival analysis, we used the Kaplan-Meier method and long-rank test was used to compare the survival plots. The chi-square test was performed

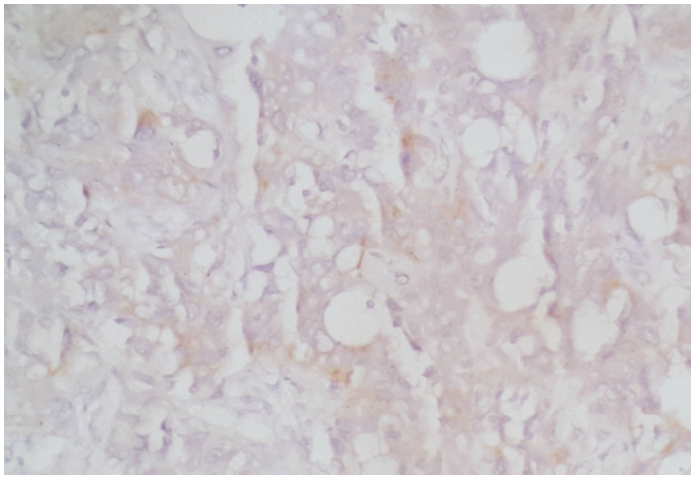

Figure 1. Representative microscopic image for the weakly positive expression of IHC staining of B7H4 (original magnification x200).

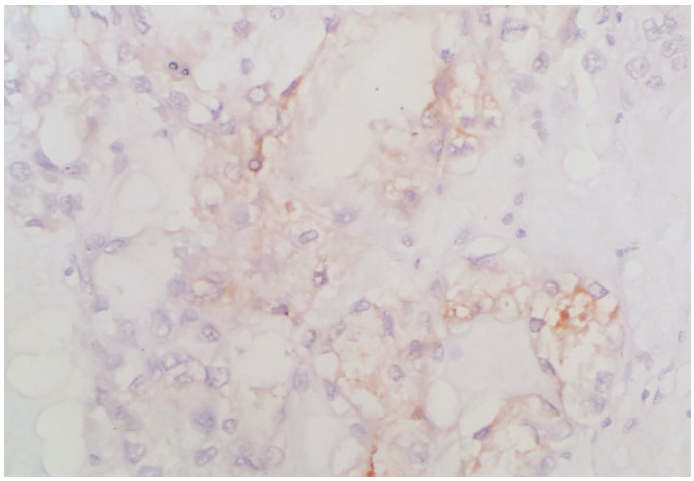

Figure 2. Representative microscopic image for the moderately positive expression of IHC staining of B7H4 (original magnification x200).

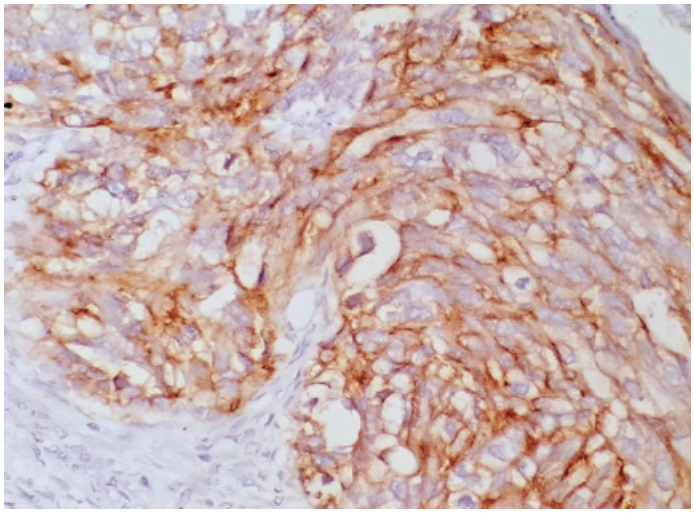

Figure 3. Representative microscopic image for the strong positive expression of IHC staining of B7H4 (original magnification x200).

to compare categorical variables and the Student's t-test for unpaired data. We employed the Cox regression analysis in order to identify factors which affect survival, presented as hazard ratios (HRs) and 95% confidence interval (CI), unadjusted or adjusted for all factors. In the multivariate analysis, we included each variable with a p value < 0.05 in the univariate analysis. For the statistical analysis, we used SPSS software version 23.0 (SPSS, Inc., Chicago, IL). We considered the p value < 0.05 as statistically significant.

RESULTS

Thirty-one patients who were diagnosed with ovarian CCC and had paraffin embedded tissue blocks, were included inthe study. Table 1 shows 31 patients' demographic and clinicopathologic characteristics.The median age of patients was 54 years (range, 31-78) and the median follow-up time was 46 months (range,4-134). Maximal and optimal CRS rates were 45.2% and 54.8%, respectively. The 5-year DFS and OS rates were 55.6% and 56.6% for entire cohort, respectively. 10 out of 31 patients died in the follow-up period; and at the end of the study period, the remaining 21 of patients were alive.

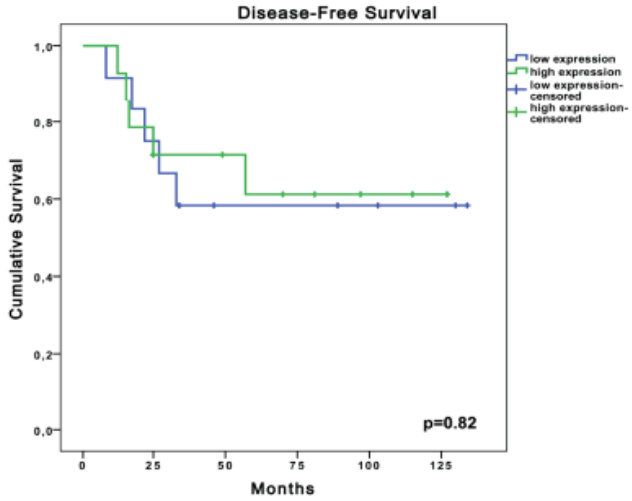

Figure 4. Disease-free survival plot of the patients according to the B7H4 expression levels

Univariate analysis revealed presence of LN metastasis (p<0.001), bilaterality (p=0.002), positive peritoneal cytology (p=0.01), presence of lymphovascular space invasion (LVSI) (p=0.009), advanced stage (p=0.02), presence of omental metastasis (p=0.02), optimal CRS (p=0.01) and postmenopausal status (p=0.04) as significant factors for decreased DFS. Postmenopausal status (HR 12.6, 95% CI: 31.20-131.91; p=0.03) and bilaterality (HR 7.92, 95% CI: 1.34-46.75; p=0.02) were defined as independent prognostic factors for DFS (Table 2) in multivariate analysis. For decreased OS, univariate analysis revealed presence of LN metastasis (p=0.004), positive cytology (p=0.02), bilaterality (p=0.01), presence

Table 1. Demographic and clinicopathologic characteristics of patients	
Characteristics	**Values, n=31 (%)**
Age, y (median, range)	54 (31-78)
Menopausal status	
Premenopausal	9 (29.0%)
Postmenopausal	22 (71.0%)
Tumor size, cm (median, range)	10 (4-29)
Ca 125, IU/ml (median, range)	99.0 (6-1195)
Stage	
I	13 (41.9%)
II	3 (9.7%)
III	14 (45.2%)
IV	1 (3.2%)
LVSI	
Absent	20 (64.5%)
Present	11 (35.5%)
Omentum metastasis	
Absent	21 (67.7%)
Present	10 (32.3%)
Peritoneal cytology	
Negative	18 (58.1%)
Positive	13 (41.9%)
Adnexal involvement	
Unilateral	22 (71.0%)
Bilateral	9 (29.0%)
Number of LNs removed (median, range)	
Total LNs	60 (22-105)
Pelvic LNs	44 (17-71)
Para-aortic LNs	18 (1-57)
LN metastasis	
Absent	19 (61.3%)
Present	12 (38.7%)
Endometriosis	
Absent	20 (64.5%)
Present	11 (35.5%)
Ascites	
Absent	23 (74.2%)
Present	8 (25.8%)
B7H4 expression	
Low	17 (54.8%)
High	14 (45.2%)
CRS	
Maximal	14 (45.2%)
Optimal	17 (54.8%)
Status	
Alive	21 (67.7%)
Dead	10 (32.3%)
Follow up, months (median, range)	46 (4-134)

Abbreviations: n: Number; LN: Lymph node; y: year; LVSI: Lymphovascular space invasion; CRS: cytoreductive surgery

Table 2. Univariate and multivariate analyses for disease free survival in the entire cohort (n=31)

	Univariate analyses of DFS* n of events (%)	p	Multivariate analysis of DFS* HR	95% CI	p
Age, y					
<54	6/15(53.3%)	0.97			
≥54	7/16(55.0%)				
Ca 125, IU/ml					
<35	2/7 (71.4%)	0.43			
≥35	11/24 (50.8%)				
Tumor diameter, cm					
<10	7/15 (47.4%)	0.64			
≥10	6/16 (61.9%)				
Endometriosis					
Absent	11/20 (44.4%)				
Present	2/11 (79.5%)	0.07			
Omentum metastasis					
Absent	6/21 (70.2%)				
Present	7/10 (30.0%)	0.02			
Stage					
I	2/13 (83.1%)				
II,III,IV	11/18 (38.1%)	0.02			
B7H4 expression					
Low	8/17 (52.9%)	0.50			
High	5/14 (61.2%)				
CRS					
Maximal	2/14 (84.4%)				
Optimal	11/17 (35.3%)	0.01			
Peritoneal cytology					
Negative	4/18 (76.9%)				
Positive	9/13 (28.8%)	0.01			
LVSI					
Absent	5/20 (70.5%)				
Present	8/11 (27.3%)	0.009			
LN metastasis					
Absent	4/19 (74.6%)				
Present	9/12 (25.0%)	<0.001			
Menopausal status					
Premenopausal	1/9 (88.9%)				
Postmenopausal	12/22 (43.1%)	0.04	12.6	31.20-131.91	0.03
Bilaterality					
No	6/22 (69.0%)				
Yes	7/9 (22.2%)	0.002	7.92	1.34-46.75	0.02

*:5-year disease free survival rate
Abbreviations: DFS: Disease Free Survival; LN: Lymph node; LVSI: Lymphovascular space invasion; HR: Hazard ratio; CI: Confidence interval; y: Year; n: Number; CRS: cytoreductive surgery

Table 3. Univariate and multivariate analyses for overall survival in the entire cohort (n=31)

	Univariate analyses of OS* n of events (%)	p	Multivariate analysis of OS* HR	95% CI	p
Age, y					
<54	6/15 (48.8%)	0.96			
≥54	6/16 (60.2%)				
Menopausal status					
Premenopausal	1/9 (83.3%)	0.05			
Postmenopausal	11/22 (46.2%)				
Ca 125, IU/ml					
<35	2/7 (68.6%)	0.45			
≥35	10/24 (53.2%)				
Tumor diameter, cm					
<10	6/15 (53.9%)	0.58			
≥10	6/16 (59.6%)				
Endometriosis					
Absent	10/20 (48.8%)	0.13			
Present	2/11 (74.1%)				
Omentum metastasis					
Absent	6/21 (67.0%)				
Present	6/10 (40.0%)	0.07			
B7H4 expression					
Low	8/17 (47.2%)	0.35			
High	4/14 (67.5%)				
Stage					
I	2/13 (83.1%)				
II,III,IV	10/18 (42.4%)	0.04			
CRS					
Maximal	2/14 (84.4%)				
Optimal	10/17 (41.2%)	0.03			
Peritoneal cytology					
Negative	4/18 (73.5%)				
Positive	8/13 (35.9%)	0.02			
LVSI					
Absent	5/20 (68.8%)				
Present	7/11 (36.4%)	0.02			
LN metastasis					
Absent	4/19 (74.6%)				
Present	8/12 (29.2%)	0.004			
Bilaterality					
No	5/22 (70.8%)				
Yes	7/9 (22.2%)	0.001	4.26	1.13-16.05	0.03

*:5-year overall survival rate
Abbreviations: OS: Overall Survival; LN: Lymph node; LVSI: Lymphovascular space invasion; HR: Hazard ratio; CI: Confidence interval; y: Year; n: Number; CRS: cytoreductive surgery

Table 4. Demographic and clinicopathologic characteristics of patients with regards to expression levels

	Low expression n =17 (%)	High expression n=14 (%)	p
Age, y			
<54	8 (47.1%)	7 (50.0%)	0.57
≥54	9 (52.9%)	7 (50.0%)	
Menopausal status			
Premenopausal	6 (35.3%)	3 (21.4%)	0.45
Postmenopausal	11 (64.7%)	11 (78.6%)	
Tumor size, cm			
<10	7 (41.2%)	8 (33.3%)	0.47
≥10	10 (58.8%)	6 (66.6%)	
Ca 125, IU/ml			
<35	5 (29.4%)	2 (14.3%)	0.41
≥35	12 (70.6%)	12 (85.7%)	
Stage			
I	5 (29.4%)	8 (33.3%)	0.15
II, III,IV	12 (70.6%)	6 (66.6%)	
LVSI			
Absent	11(64.7%)	9 (64.3%)	0.63
Present	6 (35.3%)	5 (35.7%)	
Omentum metastasis			
Absent	12 (70.6%)	9 (64.3%)	0.50
Present	5 (29.4%)	5 (35.7%)	
Peritoneal cytology			
Negative	9 (52.9%)	9 (64.3%)	0.71
Positive	8 (47.1%)	5 (35.7%)	
Adnexal involvement			
Unilateral	11 (64.7%)	11 (78.6%)	0.45
Bilateral	6 (35.3%)	3 (21.4%)	
LN metastasis			
Absent	9 (52.9%)	10 (71.4%)	0.46
Present	8 (47.1%)	4 (28.6%)	
Endometriosis			
Absent	10 (58.8%)	10 (71.4%)	0.70
Present	7 (41.2%)	4 (28.6%)	
CRS			
Maximal	7 (41.2%)	7 (50.0%)	0.72
Optimal	10 (58.8%)	7 (50.0%)	
Number of LNs removed (median, range)			
Total LNs	60 (22-105)	58.5 (22-97)	0.82
Pelvic LNs	44 (18-71)	42.5 (17-67)	0.65
Para-aortic LNs	16 (2-57)	18.5 (1-46)	0.92
Follow up, months (median, range)	43.0 (4-134)	63.5 (24-127)	0.27

Abbreviations: n: Number; LN: Lymph node; y: year; LVSI: Lymphovascular space invasion; CRS: cytoreductive surgery

of LVSI (p=0.02) and optimal CRS (p=0.03) were as significant prognostic factors. Bilaterality (HR 4.26, 95% CI:1.13-16.05; p=0.03) was the sole independent prognostic factor which was associated with decreased OS in the multivariate analysis (Table 3).

All tumor specimens had positive IHC staining with B7H4 antibody to some extent. Of the 31 patients, 14 (45.2%) showed high expression while the remaining the 17 (54.8%) had low expression with B7H4. The expression levels of B7H4 and clinicopathological characteristics of patients (Table 4) were not significantly different. The low expression group's 5-year DFS rate was 52.9% and this rate was 61.2% in the high expression group (p=0.50) (Figure 4). It was possible to compare the 5-year OS rates between the high expression group and the low expression group (67.5% and 47.2%, p=0.35; respectively) (Figure 5).

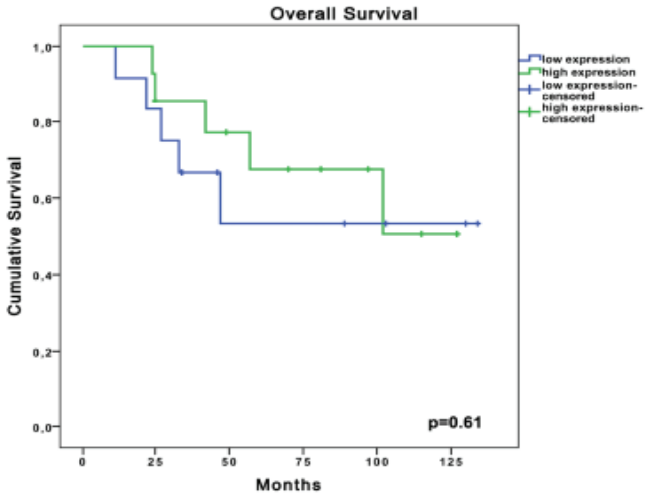

Figure 5. Overall survival plot of the patients according to the B7H4 expression levels

DISCUSSION

In the current study, all tumor specimens had positive IHC staining with B7H4 antibody to some extent. Of the 31 patients, 14 (45.2%) had high expression and the remaining had low expression with B7H4. In addition, we concluded that B7H4 expression levels were unrelated with clinicopathologic characteristics and survival outcomes of the patients.

Recently, immunotherapy, especially anti-programmed cell death ligand-1 (B7H1), has been shown to be an effective treatment in gynecological cancers, in addition to other treatment modalities (18,19). B7H4 which is a member of the B7 protein family, regulates T-cell immunologic response in a negative way by means of inhibiting cell proliferation, cell cycle progression and cytokine production (2). Blocking of B7H4 may increase the T-cell mediated antitumor immunity in B7H4 positive cancers. Therefore, B7H4 protein is considered to be a viable target for immunotherapy.

Xu et al. (9) evaluated the association between B7H1 and B7H4 expressions with clinical and pathological characteristics in 112 patients who had EOC (93 cases with serous cystadenocarcinoma, 4 with clear cell carcinoma, 3 with endometrioid adenocarcinoma and 12 cases with mucinous cystadenocarcinoma) in their study. According to their findings, there was a significant correlation between the expression levels of B7H4 with histological sub-type, disease stage, tumor size and tumor metastasis (p<0.05). However we could not demonstrate significant correlation between clinicopathologic characteristics of patients and B7H4 expression levels in our study. In the Xu study (9), there were only four ovarian CCC patients and all of these patients had low B7H4 expression. Whereas 14 (45.2%) out of the 31 patients in our study showed high expression and the remaining 17 (54.8%) had low expression with B7H4.

In another study, which was aimed to assess the expression levels of B7H4 protein in primary EOC, metastatic EOC, low malignant tumors, cystadenomas and endometriosis, the authors showed that B7H4 protein expression was found in 15/15 (100%) ovarian CCC in a predominantly cytoplasmic and circumferential membranous distribution pattern (14). The corresponding figure was 100% (31/31) in our study which was in agreement with the previous study.

Some previous studies showed that elevated B7-H4 expression by tumor cells blocks T-cell activation; therefore, it has been postulated to play a role in shielding cancer cells from immune surveillance and eluding apoptotic programs (4-6). Pagnotti et al. concluded that the density of B7H4 staining was generally weak [interquartile range (IQR) = 0.2 to 22.1] in endometrioid carcinomas, intermediate (27.7; IQR, 0.5 to 87.2) in serous carcinomas, and very strong (73.466; IQR, 44.6 to 125.0) in ovarian CCC. They also found that intensity of B7H4 staining in ovarian CCC tissue sections was inversely correlated with the number of tumor infiltrating T-cells and macrophages (10). In our study, high expression was noted in 14 out of 31 patients (45.2%) but we were unable to demonstrate an association between number of tumor infiltrating T-cell and macrophages, and B7H4 expression.

Liang et al. (13). Investigated B7H4 expression in 360 women with ovarian serous carcinoma. They found that 91% (267/293) of the HGSC and 69% (9/13) of the low grade serous ovarian carcinomas expressed B7H4. Furthermore, B7H4 expression in HGSC was associated with tumor stage (p<0.001) but not OS and DFS in their study (13). Similarly, we could not find a relationship between survival outcomes and B7H4 expression. B7H4 expression rate in ovarian CCC in our study was higher when compared to B7H4 expression rate in ovarian HGSC in their study (100% vs 91%, respectively).

In a meta-analysis in 2017, ten studies including 1045 patients with ovarian cancer were evaluated (20). The meta-analysis concluded that there was a significantly-increased B7H4 expression in ovarian cancer patients [Odds ratio (OR) 4.20, 95% CI: 2.85-6.18, p< 0.05]. In accordance with the clinicopathologic features, there was no association found between B7H4 expression and FIGO stages (OR 0.81, 95% CI: 0.64-1.03, p=0.09), tumor metastasis (OR 1.25, 95% CI: 0.90-1.74, p=0.18),

grade (OR 0.91, 95%CI: 0.72-1.16, p= 0.45) or histologic subtypes (OR 1.17, 95% CI:0.85-1.60, p=0.34) in ovarian cancer (20). However, there was a significant association between B7H4 expression and a worse progression free survival (HR 1.30, 95% CI: 1.17-1.45, p<0.05) (20). We demonstrated that B7H4 was overexpressed in ovarian CCC but B7H4 expression levels were unrelated with clinicopathologic characteristics and survival outcomes of the patients.

This study is mostly limited to inherent heterogeneity of tissue specimens. We tried to overcome this potential problem by obtaining data from five separate histologic regions for each case. The B7H4 expression score was based on subjective evaluation of the pathologist. However, all tumor specimens were evaluated by the same gynecopathologist to minimize potential diagnostic bias across the specimens.

CONCLUSION

In conclusion, we concluded that B7H4 expression is a biomarker for ovarian CCC, but it is unrelated with clinicopathologic characteristics and survival outcomes of patients. Therefore, it can be speculated that B7H4 protein may be a potential immunotherapeutic target for patients who have ovarian CCC.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: Date and number: 28.03.2019/26.

Zeliha Firat Cuylan ORCID: 0000-0003-3382-8763

Koray Aslan ORCID: 0000-0002-3432-781

Kamil Hakan Muftuoglu ORCID: 0000-0003-4069-0002

Murat Oz ORCID: 0000-0002-0629-5386

Mehmet Mutlu Meydanli ORCID: 0000-0001-6763-9720

REFERENCES

1. Cheng C, Qu QX, Shen Y, et al. Overexpression of B7-H4 in tumor infiltrated dendritic cells. J Immunoassay Immunochem 2011;32:353-64.
2. He C, Qiao H, Jiang H, et al. The inhibitory role of b7-h4 in antitumor immunity: association with cancer progression and survival. Clin Dev Immunol 2011;2011:695834.
3. Carreno BM, Collins M. The B7 family of ligands and its receptors: new pathways for costimulation and inhibition of immune responses. Annu Rev Immunol 2002;20:29-53.
4. Wang X, Wang T, Xu M, et al. B7-H4 overexpression impairs the immune response of T cells in human cervical carcinomas. Hum Immunol 2014;75:1203-9.
5. Kryczek I, Wei S, Zou L, et al. Cutting edge: induction of B7-H4 on APCs through IL-10: novel suppressive mode for regulatory T cells. J Immunol 2006;177:40-4.
6. Chen C, Qu QX, Shen Y, et al. Induced expression of B7-H4 on the surface of lung cancer cell by the tumor-associated macrophages: a potential mechanism of immune escape. Cancer Lett 2012;317:99-105.
7. Seliger B, Quandt D. The expression, function, and clinical relevance of B7 family members in cancer. Cancer Immunol Immunother 2012;61:1327-41.
8. Salceda S, Tang T, Kmet M, et al. The immunomodulatory protein B7-H4 is overexpressed in breast and ovarian cancers and promotes epithelial cell transformation. Exp Cell Res 2005;306:128-41.
9. Xu M, Zhang B, Zhang M, et al. Clinical relevance of expression of B7-H1 and B7-H4 in ovarian cancer. Oncol Lett 2016;11:2815-9.
10. Pagnotti GM, Atkinson RM, Romeiser J, et al. B7-H4 is Inversely Correlated With T-Cell Infiltration in Clear Cell but Not Serous or Endometrioid Ovarian Cancer. Appl Immunohistochem Mol Morphol 2017;27:515-22.
11. Maskey N, Li K, Hu M, et al. Impact of neoadjuvant chemotherapy on lymphocytes and co-inhibitory B7-H4 molecule in gastric cancer: low B7-H4 expression associates with favorable prognosis. Tumour Biol 2014;35:11837-43.
12. Xu Y, Zhu S, Song M, et al. B7-H4 expression and its role in interleukin-2/interferon treatment of clear cell renal cell carcinoma. Oncol Lett 2014;7:1474-8.
13. Liang L, Jiang Y, Chen JS, et al. B7-H4 expression in ovarian serous carcinoma: a study of 306 cases. Hum Pathol 2016;57:1-6.
14. Tringler B, Liu W, Corral L, Torkko KC, Enomoto T, Davidson S, et al. B7-H4 overexpression in ovarian tumors. Gynecol Oncol 2006;100:44-52.
15. Sugiyama T, Kamura T, Kigawa J, Terakawa N, Kikuchi Y, Kita T, et al. Clinical characteristics of clear cell carcinoma of the ovary: a distinct histologic type with poor prognosis and resistance to platinum-based chemotherapy. Cancer 2000;88:2584-9.
16. Kurman RJ, Carcangiu, ML, Herrington CS, et al. WHO Classification of Tumours of Female Reproductive Organs. Fourth Edition. Lyon. IARC 2014;Chapter 1: Tumors of the ovary.
17. Prat J, Belhadj H, Berek J, et al. Abridged republication of FIGO's staging classification for cancer of the ovary, fallopian tube, and peritoneum. Eur J Gynaecol Oncol 2015;36:367-9.
18. Pan K, Gong J, Huynh K, et al. Current Systemic Treatment Landscape of Advanced Gynecologic Malignancies. Target Oncol 2019.
19. Matulonis UA, Shapira-Frommer R, Santin AD, et al. Antitumor Activity and Safety of Pembrolizumab in Patients with Advanced Recurrent Ovarian Cancer: Results from the Phase 2 KEYNOTE-100 Study. Ann Oncol 2019.
20. Meng Z, Wang F, Zhang Y, et al. B7-H4 as an independent prognostic indicator of cancer patients: a meta-analysis. Oncotarget 2017;8:68825-36.