Expression of V-domain immunoglobulin suppressor of T cell activation is associated with the advanced stage and presence of lymph node metastasis in ovarian cancer

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Abstract. V-domain immunoglobulin suppressor of T cell activation (VISTA) is a novel negative immune checkpoint that belongs to the B7 family. VISTA is primarily expressed on hematopoietic cells and inhibits T cell proliferation and cytokine production. The blockade of VISTA has demonstrated promising results in certain murine tumor models. In the present study, an immunohistochemical analysis of VISTA expression on tumor cells, intratumoral immune cells and vascular endothelial cells was performed in a cohort of 65 patients with ovarian cancer (OC). The associations between VISTA expression and different clinicopathological characteristics were evaluated using Fisher's exact test, and the analysis of overall survival in different groups was performed by the construction of Kaplan-Meier curves. The results indicated that high expression of VISTA on tumor cells or ICs was significantly associated with advanced tumor stage and the presence of lymph node metastasis (LNM). However, the percentage of cases with high expression of VISTA on tumor cells (24.6%) was decreased compared with those with high expression on ICs (44.6%). There was no association between VISTA expression and the 5-year overall survival rate, and advanced-stage disease was the only independent predictor of poor prognosis based on multivariate Cox regression analysis. In general, VISTA expression increased with advanced disease stage and LNM, indicating that VISTA expression is involved in the progression of OC. More importantly, these data implicate VISTA as a candidate immunotherapeutic target in OC.

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Abbreviations: VISTA, V-domain immunoglobulin suppressor of T cell activation; OC, ovarian cancer; PD-1, programmed cell death 1; IS, immunoreactivity score; ICs, immune cells; VECs, vascular endothelial cells; LNM, lymph node metastasis

Key words: V-domain immunoglobulin suppressor of T cell activation, immunotherapy, immune checkpoint, ovarian cancer, overall survival analysis, immunohistochemical staining

Introduction

Ovarian cancer (OC) represents a diverse group of malignant diseases that arise from epithelial cells, stromal cells or ova, and even from the fallopian tube and endometrium (1). Among these forms of OC, epithelial ovarian cancer accounts for 90% (2). According to histopathological classification, almost 75% of OC cases are serous and 3% of all OC cases are mucinous (3). As 70% of cases are diagnosed at an advanced stage, the fatality-to-case ratio of OC is high, even following surgical debulking and adjuvant chemotherapy (4). It is the most lethal gynecologic disease, with a 5-year survival rate of only 50%. OC affected 22,280 females in the United States of America in 2016 and caused 14,240 mortalities according to a report from the National Cancer Institute (National Institutes of Health, Bethesda, MA, USA) (5).

Although OC is one of the most chemo-sensitive types of solid tumors and is associated with a high initial response, chemoresistance and recurrence of OC are serious problems associated with the current treatments (6,7). Therefore, novel and effective therapies for OC are urgently required. Cancer immunotherapy involves utilizing the immune system of the patient to attack tumor cells by targeting tumor-specific antigens. These strategies, which include therapeutic vaccines, immunomodulators, immune checkpoint inhibitors and adoptive T cell transfer, have yielded breakthroughs in the treatment of certain types of cancer (8,9). OC is an ideal candidate for immunotherapy due to the good performance of immunoregulatory cells including T helper cells, the short average duration of the decrease in the number of immunoregulatory cells following standard cytotoxic therapy and the satisfactory nutritional status of the patients even in the late course of OC; however, in general, immune-based OC therapies have only been modestly successful (8-10). Previously, the successful performance of immune checkpoints, including the programmed cell death 1 (PD-1) receptor, has attracted

attention in the search for novel treatments for certain types of cancer (11).

V-domain immunoglobulin suppressor of T cell activation (VISTA) is a novel negative immune checkpoint regulator that is homologous to programmed cell death ligand 1 (PD-L1) (12). VISTA is highly expressed on hematopoietic cells, with the greatest densities in myeloid and granulocytic cells, and weaker expression on cluster of differentiation (CD)4+ and CD8+ T cells (13). Similar to PD-L1, VISTA functions to inhibit T cell activation to maintain tolerance and limit immunopathology (14). The inhibition of VISTA weakens the suppressive function of T cells, resulting in a decrease in tumor growth (14,15). In murine fibrosarcoma models, VISTA overexpression on tumor cells was demonstrated to induce immune protection against the growth of control tumor cells (12). Additionally, the use of an anti-VISTA monoclonal antibody in murine cancer models was suggested to impair tumor growth, with particularly marked results when used in combination with a tumor vaccine (12). These observation indicate that VISTA is a promising target in cancer therapy (16).

However, to the best of our knowledge, the VISTA expression in OC and evidence for an association between VISTA and OC has not yet been demonstrated. Therefore, in the present study, the expression VISTA in tumor tissues samples from patients with OC at different stages was examined, and the prognostic value of VISTA in different types of OC was evaluated.

Materials and methods

Patients and tissue samples. In this retrospective study, archived formalin-fixed paraffin-embedded OC specimens from 65 patients with OC treated between June 2006 and June 2012 were obtained from the Pathology Department of West China Second University Hospital, Sichuan University (Chengdu, China). Patients were included based on the following criteria: i) Accessible clinical data and at least 5 years of routine follow-ups; ii) no chemotherapy or radiation therapy prior to oophorectomy; and iii) OC confirmed by histopathological diagnosis. The characteristics of patients, including age (age range, 19-80 years; median age, 53 years) and stage of OC, are summarized in Table I.

The present study was approved by the Ethics Committee of West China Second Hospital of Sichuan University and informed consent was obtained from all patients undergoing surgery.

Immunohistochemistry (IHC). VISTA expression in OC tissues was analyzed immunohistochemically. The OC tissues were fixed in 10% (v/v) formalin at room temperature for 48 h once being removed from the patients during surgery, and embedded in paraffin until use. The paraffin-embedded tissues were sectioned (thickness, 3-4 μm) and mounted on poly-l-lysine-coated slides. Firstly, samples were incubated at 37˚C overnight prior to being deparaffinized with 99% (v/v) xylene and sequentially rehydrated in a graded ethanol series (100, 95, 80 and 50%). Slides were then rinsed twice with PBS containing 0.1% Tween-20 (PBST). High-temperature antigen retrieval was performed using 10 mmol/l boiling (~95˚C) sodium citrate buffer at pH 6.0 for 15 min. To block the endogenous peroxidase activity, samples were immersed in 3% hydrogen peroxide for 30 min at room temperature, followed by incubation in 5% bovine serum albumin (BSA) (cat. no. 9048-46-8; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 30 min to reduce non-specific binding. Slides were then incubated with a primary monoclonal rabbit anti-human VISTA antibody (cat. no. 64953, Cell Signaling Technology, Inc., Danvers, MA, USA; 1:50 dilution in 5% BSA) at 4˚C overnight. Following rinsing in PBST 3 times for 5 min each, slides were incubated with a secondary horseradish peroxidase-conjugated goat anti-rabbit IgG (cat. no. A0208; Beyotime Institute of Biotechnology, Haimen, China) for 1 h at room temperature. Slides were then rinsed thoroughly in PBST 3 times for 5 min each prior to incubation with streptavidin peroxidase for 30 min at room temperature. Subsequent to thorough rinsing with PBST three times, slides were then incubated with 1% (w/v) 3,3'-diaminobenzidine solution to develop color for 10 min at room temperature. Finally, slides were counter-stained with 0.5% (w/v) hematoxylin at room temperature for 5 min and mounted with neutral balsam prior to being examined under a Leica DM1000 light microscope at a magnification of x400 (Leica Microsystems GmbH, Wetzlar, Germany). Stained cell cytoplasm was considered to indicate positivity for VISTA expression, and specimens from healthy ovarian tissues were used as controls. The healthy controls (age range, 41 to 56; median age, 47.4) in the present study were females from West China Second University Hospital, Sichuan University with benign gynecological diseases including uterine myoma or mesosalpinx cysts. Small pieces of normal ovarian tissues were obtained subsequent to provision of written informed consent prior to laparoscopic surgery. Patients were fully informed of the disadvantages of the procedure and the applications of the tissues prior to surgery.

Evaluation of VISTA protein expression. For evaluation of VISTA protein expression in OC tissues, a reproducible semi-quantitative method that considered the staining intensity (regardless of the positive subcellular location) and numbers of positive tumor cells was adopted as described previously (17,18). In brief, the VISTA staining intensity was classified as follows: 0, negative staining; 1, weak staining (light yellow); 2, moderate staining (yellow-brown); and 3, strong staining (brown). In the same tumor tissue with different staining intensities, only the highest intensity was recorded. The percentage of VISTA-positive cells was also scored as follows: 0, no stained cells; 1, 1-30% positive cells; 2, 31-60% positive cells; 3, 61-90% positive cells; 4, 91-100% positive cells. The final immunoreactivity score (IS) of each sample was calculated by adding the scores for the staining intensity and the percentage of VISTA-positive cells. Scores of 0-3 were defined as 'negative expression' (-), scores of 4-5 as 'weakly positive expression' (+), and scores of 6-7 as 'strongly positive expression' (++). In addition, overall scores were dichotomized into two groups: Low expression (IS <5); and high expression (IS ≥5) in OC samples.

The proportion of VISTA-positive immune cells (ICs)/200 ICs in the intratumoral hotspot regions, where the highest density of VISTA-positive ICs accumulated, was considered

in the analysis, as described previously (19). Patients with OC with ≤35 VISTA-positive ICs were classified as exhibiting low VISTA expression in terms of the proportion of VISTA-positive ICs/200 ICs. Immunostaining of vascular endothelial cells (VECs) was graded as present or absent. Each sample was scored by two of the authors with assitance from a pathologist (West China Second University Hospital of Sichuan University).

Statistical analysis. In the present study, patients were followed until mortality or to the end of the follow-up period (November 2012). The overall survival (OS) was calculated from the date of the initial diagnosis to the date of mortality or the last follow-up. Patients were censored at the date of the last visit or at the time of mortality due to non-OC-associated causes.

The correlation between clinicopathological characteristics and VISTA expression in OC was analyzed using the Pearson's χ^2 test or Fisher's exact test with SPSS v22 software (IBM Corp., Armonk, NY, USA). Kaplan-Meier 5-year survival curves were generated compared using log-rank tests to assess OS. Univariate and multivariate Cox proportional hazard models were used to estimate the associations between VISTA expression and clinical characteristics with OS. P<0.05 (two-tailed) was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 65 patients with OC (aged 19-80 years, median 53 years) were included in the present study. At the end of the 6-year study period, 33 cases of survival were censored, while the other 32 events were OC-associated mortalities. The median survival time of this group was 52.3±3.9 months (95% CI, 44.8-60.0 months) and the 5-year OS rate was 47.7% (Fig. 1). The characteristics of the patients included in the present study are summarized in Table I.

VISTA expression in normal ovarian and ovarian cancer tissues detected by IHC. As demonstrated in Fig. 2, brown positive immunostaining for VISTA was observed in the tumor cells, ICs and VECs in the OC tissues. The final IS of each sample was based on a combination of the staining intensity [ranging from negative (0) to strong (3) (median, 0)] and the percentage of VISTA-positive cells [ranging from 0 to 95% (median, 1)]. VISTA-positive tumor cells were detected in 20/65 patients (30.8%). These cells were primarily located in the adenoid structure of the tumor lesions. Only 16/65 cases (24.6%) were defined as high VISTA expression (IS ≥5). Overall, the percentage of positive tumor cells and the staining intensity were low in the ovarian adenocarcinoma cases in the present study.

In the majority of cases, the tumor-infiltrating ICs accumulated in the interstitial sites, which were defined as intratumoral hotspot regions. VISTA-positive cells were detected in 59 cases (90.8%), and the proportion of VISTA-positive ICs/200 ICs ranged from 5-86 (median, 33). A total of 29/65 cases (44.6%) were classified as exhibiting high expression of VISTA-positive immune cells (>35 ICs/200 ICs). In the normal ovarian tissue, several sporadic VISTA-positive ICs were also observed. In addition, VISTA-positive VEC (yellow-brown circles under light microscopy) were identified in 15 cases (23.1%).

Clinical significance of VISTA expression in OC. The results of the examination of VISTA expression in OC are summarized in Table I. VISTA expression on tumor cells was significantly increased in patients with advanced-stage OC (III+IV) compared with those with lower-stage disease (P=0.043). Furthermore, VISTA expression on tumor cells was more prevalent in cases with LNM compared with those without (P=0.015). High expression of VISTA on ICs was also associated with the tumor stage and LNM, with significantly higher frequencies of advanced stage disease (III+IV) (P=0.047) and LNM (P=0.042) among cases with a high proportion of VISTA-positive ICs/200 ICs compared with those with a low proportion. VISTA expression on VECs was only associated with LNM status, with a significantly increased frequency of VISTA-positive VECs in cases with LNM compared with those without (P=0.001). However, there were no significant associations between patient age, grade of tumor cell differentiation, histologic type of adenocarcinoma, primary therapy or residual tumor and VISTA expression on tumor cells, ICs and VECs.

Survival analysis and prognostic significance of VISTA expression in OC. To explore the potential association between VISTA expression and the prognosis of OC, OS analysis was performed by constructing Kaplan-Meier curves. As indicated in Table I, the median survival time was slightly decreased in patients with high VISTA expression either in tumor cells (49.1±7.6 vs. 53.3±4.5 months) or in ICs (46.5±5.7 vs. 55.9±5.0 months) compared with that in patients with lower VISTA expression. There was no significant difference in the 5-year OS rate of patients with high VISTA expression (n=16) in tumor cells compared with those with low VISTA expression (n=49; 37.5% vs. 48.97%; P=0.594; Fig. 1B). Similarly, there was no significant difference in the 5-year OS rates of patients with low VISTA expression on intratumor ICs compared with those with high VISTA expression (52.8 vs. 41.4%; P=0.232; Fig. 1C). Furthermore, there was no significant difference in the 5-year OS rates of patients with and without VISTA-positive VECs (52.0 vs. 40.0%; P=0.459; Fig. 1D). These results indicated that there was no association between VISTA-positive tumor cells, ICs and VECs and the prognosis of patients with OC.

The associations between the 10 clinicopathological characteristics and OS in patients with OC were evaluated using a univariate Cox regression model. The results in Table II suggested that advanced-stage (III+IV) OC [hazard ratio (HR)=2.987; P=0.008], LNM (HR =2.218; P=0.025) and the presence of residual tumor tissue following primary surgery (HR=2.192; P=0.030) were associated with poor prognosis. The role of these three factors in prognostic prediction was additionally investigated using a multivariate Cox regression model with the forward step-wise method. The results revealed that only advance-stage (III+IV) OC (HR=2.445; P=0.032) was an independent prognostic factor that may be used to predict poor survival.

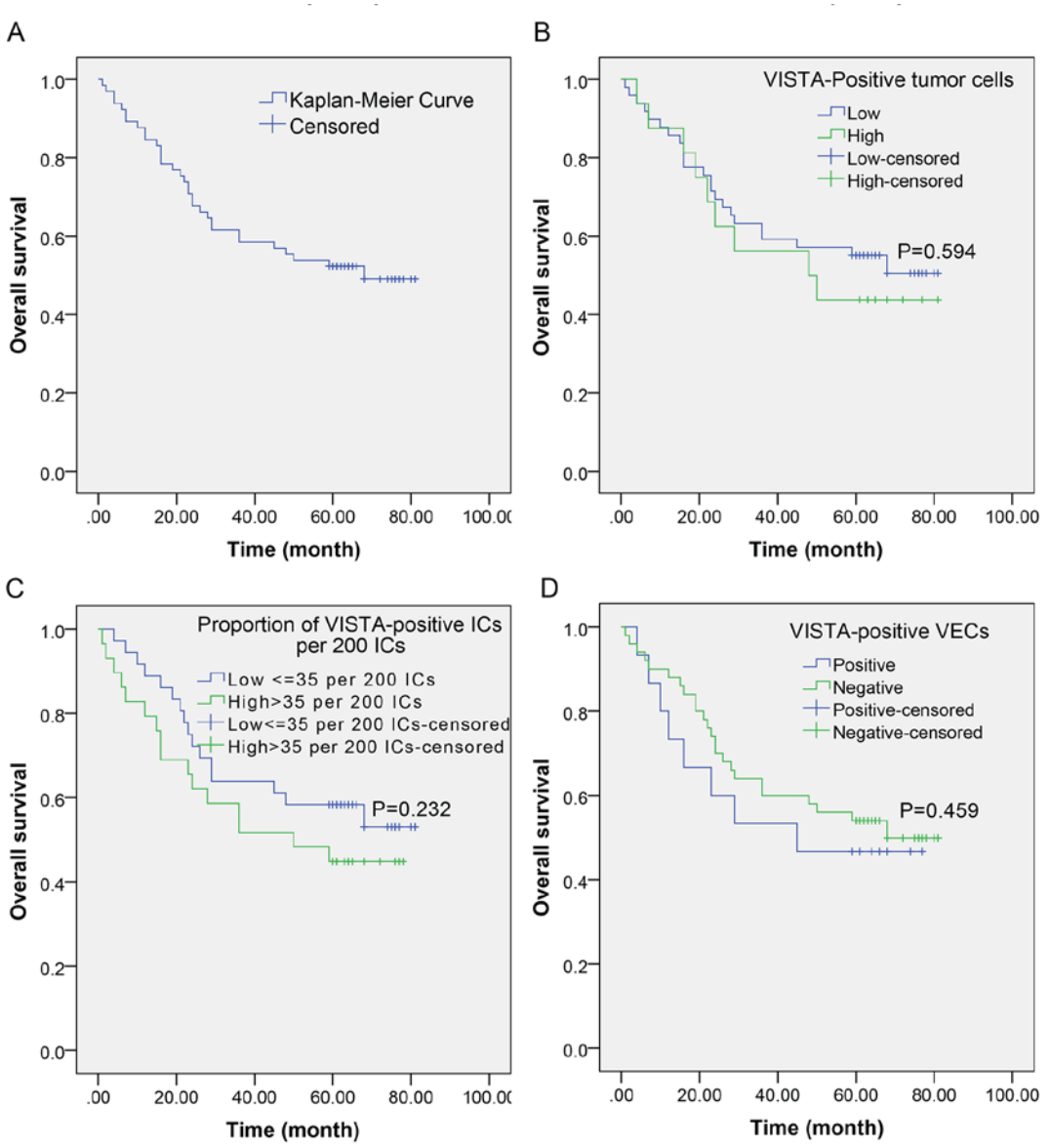

Figure 1. Prognostic significance of VISTA expression on tumor cells, ICs and VECs. (A) The median survival time of the 65 patients with OC was 52.3±3.9 months (95% confidence interval, 44.8-60.0 months) and the 5-year OS rate was 47.7%. No significant associations between tumor-specific overall survival and VISTA expression on (B) tumor cells (median survival, 49.1±7.6 vs. 53.3±4.5 months; P=0.594), (C) ICs (median survival, 46.5±5.7 vs. 55.9±5.0 months; P=0.232) or (D) VECs (median survival, 45.7±7.9 vs. 53.7±4.3 months; P=0.459) were observed, indicating that none of the factors were prognostic predictors for the overall survival in patients with ovarian cancer. VISTA, V-domain immunoglobulin suppressor of T cell activation; ICs, immune cells; VECs, vascular endothelial cells.

Discussion

VISTA is a novel immune checkpoint molecule, the prevalence of which has been demonstrated previously in a cohort of patients with human gastric carcinoma and oral squamous cell carcinoma (19,20); however, the corresponding data for human OC are presently unavailable. In the present study, the expression of VISTA in tumor cells, ICs and VECs in patients with OC with different clinicopathological characteristics was first evaluated. This information is important for improving our understanding of the role of VISTA in human OC.

Immunotherapy for various types of cancer has evolved rapidly in previous years, due to critical advances in our understanding of the immunomodulatory signaling pathways in immune cells and the tumor microenvironment (9). In particular, immune checkpoints are a group of molecules involved in the inhibitory pathways that regulate self-tolerance and modulate the duration and amplitude of physiological immune responses to heterogeneous tissues (21). Therefore, cancer immunotherapy targeting immune checkpoints, including cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), PD-1 and PD-L1, have exhibited encouraging performances in a wide range of types of cancer, particularly melanoma, and renal and lung cancer (11,22). VISTA, having homology to the B7 family ligand PD-L1, exerts its immunosuppressive activities on resting and activated human CD4+ and CD8+ T cells *in vitro* and *in vivo* (12,14). In a murine melanoma model, the blockade of VISTA alone inhibited the suppressive characteristics of the tumor microenvironment and enhanced protective

Table I. VISTA expression associated with clinicopathological characteristics in patients with ovarian cancer (n=65).

Characteristics	Total (%) 65	VISTA-positive tumor cells			VISTA-positive ICs/200 ICs			Vascular endothelial cells		
		Low, n (%) 49 (75.38)	High, n (%) 16 (24.62)	P-value	Low, n (%) 36 (55.38)	High, n (%) 29 (44.62)	P-value	Negative, n (%) 50 (76.92)	Positive, n (%) 15 (23.08)	P-value
Age, years				0.57			0.221			1
≤55	37 (56.92)	29	8		23	14		28	9	
>55	28 (43.08)	20	8		13	15		22	6	
Stage				0.043[a]			0.047[a]			0.075
I+II	27 (41.54)	24	3		19	8		24	3	
III+IV	38 (58.46)	25	13		17	21		26	12	
Grade				0.718			0.52			0.706
Low (G1+G2)	11 (16.92)	9	2		5	6		8	3	
High (G3)	54 (83.08)	40	14		31	23		42	12	
Lymph node metastasis				0.015[a]			0.042[a]			0.001[a]
Negative	42 (64.62)	36	6		28	14		38	4	
Positive	23 (35.38)	13	10		8	15		12	11	
Histologic type				0.774			0.212			0.238
Serous adenocarcinoma	26 (40.00)	19	7		17	9		20	6	
Non-serous adenocarcinoma	39 (60.00)	30	9		19	20		30	9	
Primary therapy				0.252			0.622			0.566
Surgery	4 (6.15)	2	2		3	1		4	0	
Surgery + others	61 (93.85)	47	14		33	28		46	15	
Residual tumor				0.06			0.219			0.769
Negative	35 (53.85)	28	7		22	13		26	9	
Positive	30 (46.15)	17	13		14	16		24	6	
Tumor-specific survival, months				0.594			0.232			0.459
Total/events/censored	65/32/33	49/23/26	16/9/7		36/16/20	29/16/13		15/9/6	50/23/27	
Median survival	52.3±3.9	53.3±4.5	49.1±7.6		55.9±5.0	46.5±5.7		45.7±7.9	53.7±4.3	
95% confidence interval	44.8-60.0	44.6-62.1	34.3-64.0		46.2-65.5	35.3-57.8		30.1-61.2	45.3-62.2	

VISTA, V-domain immunoglobulin suppressor of T cell activation; ICs, immune cells. [a]P<0.05. Events, cancer-associated mortality; Censored, patients were alive at the date of the last visit or at the time of mortality due to non-OC-associated causes.

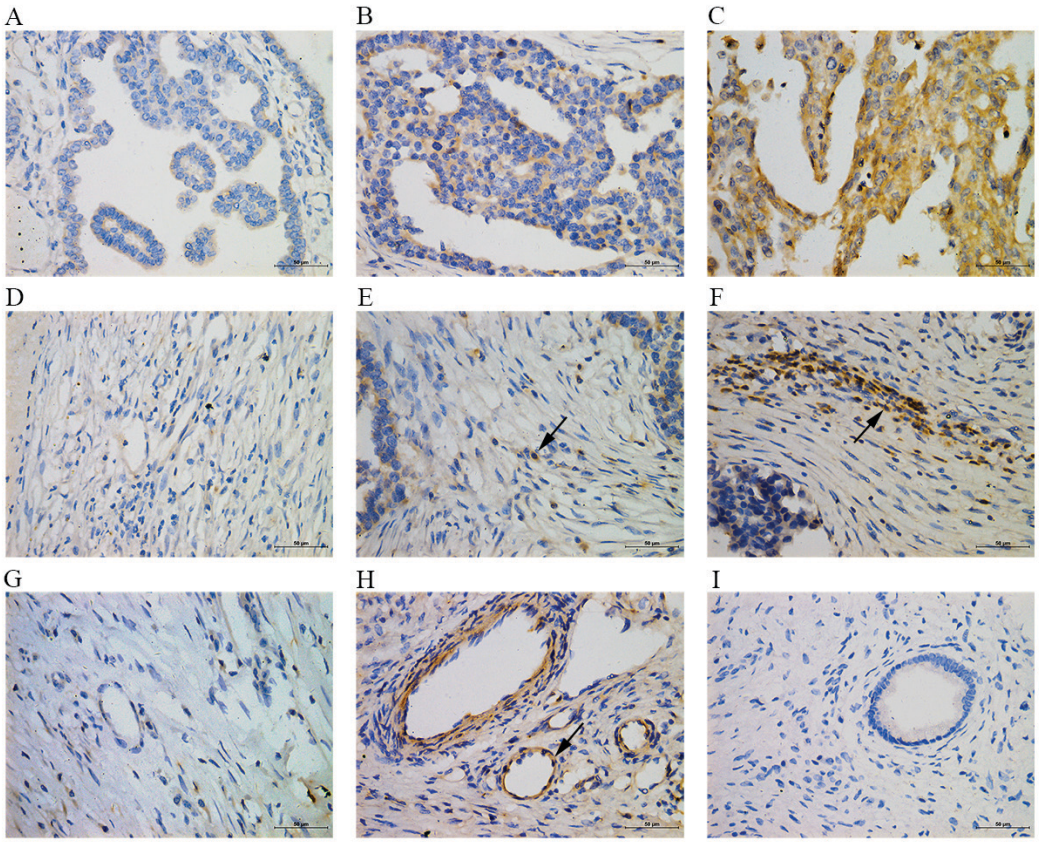

Figure 2. VISTA expression in ovarian cancer. VISTA-positive tumor cells were detected in 20/65 patients (30.8%), and staining was graded as (A) weak, (B) moderate or (C) strong. VISTA-positive immune cells were observed in 59/65 cases (90.8%), and were classified as (D) absent, (E) low and (F) high. VISTA expression on vascular endothelial cells was (G) absent in the majority of cases and (H) present in only 15 cases (23.1%). (I) Normal ovarian tissue was used as the negative control. VISTA expression is indicated by the black arrow. Scale bar, 50 μm. VISTA, V-domain immunoglobulin suppressor of T cell activation.

antitumor immunity. Furthermore, the growth of transplantable and inducible tumors was also significantly suppressed when VISTA blockade was administered concomitantly with a peptide vaccine (23).

In humans and mice, VISTA is predominantly expressed in the hematopoietic tissues, or in tissues that contain significant numbers of infiltrating leukocytes (14). Wang *et al* (12) suggested that VISTA expression was confined to the leukocytes infiltrating the tumor in a murine cancer model; however, VISTA expression on tumor cells in human gastric carcinoma has also been demonstrated (19). In accordance with these studies, tumor-infiltrating VISTA-positive ICs were easy to detect in OC tissues in the present study, with almost one-half (44.6%) defined as exhibiting high VISTA expression. Additionally, cytoplasmic VISTA expression on tumor cells was observed in OC cases, although only a small subset (24.7%) were regarded as exhibiting high VISTA expression. Notably, high VISTA expression on tumor cells and ICs was positively associated with advanced-stage OC and the presence of LNM, suggesting that VISTA is involved in OC progression. Wu *et al* (20) also identified that the expression of VISTA was associated with lymph node status in human oral squamous cell carcinoma. Activated VISTA serves a role in tumor evasion from the immune system by preventing promiscuous resting T-cell responses to self-antigens (13). Furthermore, VISTA expression was suggested to be associated with the expression of the PD-L1 in gastric cancer, indicating that VISTA cooperates with PD-L1 in the mechanism underlying immune evasion (19). Therefore, in OC, the association of advanced disease stage with high VISTA expression may be explained by the capacity of this molecule to protect VISTA-positive tumor cells or ICs from the immune responses that inhibit tumor growth and metastasis (23). VISTA-positive VECs were also detected in certain OC tissues, although no associations with any of the clinicopathological characteristics were observed.

Following the implementation of cytoreductive surgery and adjuvant chemotherapy, the 5-year survival rate of patients with OC has increased to ~50%; however, this improvement does not match the rates for thyroid or prostate cancer (5,24). Advanced stage, poor tumor differentiation and large tumor size are suggested to be associated with poor prognosis in patients with OC, and other pathological data, including the increased expression of cleaved caspase-3 and the PD-L1 in OC, have also been identified as predictive factors for OC prognosis (4,25,26). In the univariate and multivariate Cox regression analyses of the patient cohort in the present study, advanced OC stage was the only independent factor that predicted poor OS. Apart from this predictor, there was no significant association between VISTA expression and OS of patients with OC in the present study. However, due to the small cohort (n=65) of patients included in these analyses, the conclusion that VISTA expression is not involved in the progression of OC requires additional confirmation. Nevertheless, two other studies revealed that the expression

Table II. Univariate and multivariate Cox analyses for cancer-specific overall survival in patients with ovarian cancer (n=65).

		Univariate analysis		Multivariate analysis	
Characteristics	N	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years		1.260 (0.629-2.524)	0.515	-	-
≤55	37				
>55	28				
Stage		2.987 (1.329-6.717)	0.008[a]	2.455 (1.080-5.584)	0.032[a]
I+II	27				
III+IV	38				
Grade		0.781 (0.321-1.899)	0.585	-	-
Low (G1+G2)	11				
High (G3)	54				
Lymph node metastasis		2.218 (1.105-4.451)	0.025[a]	1.664 (0.819-3.380)	0.159
Negative	42				
Positive	23				
Histologic type		1.066 (0.521-2.181)	0.862	-	-
Serous adenocarcinoma	26				
Non-serous adenocarcinoma	39				
Primary therapy		0.771 (0.184-3.228)	0.721		
Surgery	4				
Surgery + others	61				
Residual tumor		2.192 (1.081-4.445)	0.030[a]	1.818 (0.890-3.713)	0.101
Negative	35				
Positive	30				
VISTA-positive tumor cells		1.241 (0.574-2.682)	0.584	-	-
Low	49				
High	16				
VISTA-positive ICs/200 ICs		1.621 (0.809-3.249)	0.173	-	-
Low	36				
High	29				
Vascular endothelial cells		1.105 (0.476-2.566)	0.817	-	-
Negative	50				
Positive	15				

VISTA, V-domain immunoglobulin suppressor of T cell activation; ICs, immune cells; HR, hazard ratio; 95% CI, 95% confidence interval. [a]P<0.05.

of VISTA alone was not associated with OS, but functioning together with CD8+ T cells in the prediction of overall survival in human oral squamous cell carcinoma (20).

Although there was no association between VISTA expression and OS, positive VISTA expression increased with advanced stage, indicating a potential role of VISTA in OC progression. Therefore, it may be speculated that VISTA represents a candidate biomarker of advanced tumor stage in OC. More importantly, it has been demonstrated that OC is an immunogenic tumor that induces a spontaneous antitumor immune response (27). Therefore, VISTA is also implicated as a potential target for OC immunotherapy. Although the mechanisms of immunotherapy targeting immune checkpoints, including PD-L1 and CTLA-4, remain to be defined, initial results appear promising (28,29). A particular challenge in the application of immunotherapy in OC is the identification of the patients who will benefit from the immune checkpoint therapy. Therefore, the measurement of VISTA expression in the tumor tissue may be a potential biomarker used to evaluate patients for inclusion in VISTA-associated therapy and contribute to the development of personalized treatment programs.

In conclusion, VISTA-positive tumor cells, ICs and VECs were detected in OC tissues. In addition, VISTA expression on tumor cells and ICs was associated with advanced OC stage and the presence of LNM, suggesting that this immune checkpoint molecule may be involved in the progression of OC. In the present study, advanced stage (III+IV) was identified as an independent prognostic factor for the prediction of poor survival in OC. Although unsuitable as a prognostic marker of

OC, VISTA represents a potential biomarker for selection of patients for VISTA-associated therapy in the future.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HL performed case collecting, experiments, specimen observing, data analysis and paper writing. HZ analysed the specimens and collected data. HW performed data analysis. SL designed the study, performed case collecting and assisted with writing the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of West China Second Hospital of Sichuan University and informed consent was obtained from all patients undergoing surgery and control patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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