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Original Article

VCAM1 expression correlated with tumorigenesis and poor prognosis in high grade serous ovarian cancer

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Abstract: High expression of vascular cell adhesion molecule 1 (VCAM1) has been shown to be associated with several cancers although its role in ovarian cancer development is largely undefined. The purpose of this study is to investigate its role in ovarian cancer using the epithelial cells and ovarian cancer cell lines and correlate its expression with clinicopathologic parameters in ovarian cancer patients. VCAM1 expression was examined via immunohistochemical staining of 251 high grade serous carcinoma samples using tissue microarray. The expression of VCAM1 was silenced in RAS-transformed ovarian epithelial cell lines and two high grade ovarian cancer cell lines. Cell migration was analyzed in vitro and effect on tumor growth was analyzed in nude mice. High VCAM1 expression was found to be was related with response to surgery and chemotherapy drugs (P = 0.025) and elder age at diagnosis (P = 0.008). Cox regression multivariable analysis showed that VCAM1 expression in tumor cells was an independent prognostic factor. Ovarian cancer cells with VCAM1 overexpression, compared with corresponding control cells, had increased cell migration and enhanced growth of xenograft tumors in mice. Our data provide strong evidence that VCAM1 plays an important role in ovarian tumor growth, and it may be used as a prognostic factor and novel therapeutic target for ovarian cancer.

Keywords: Ovarian cancer, VCAM1, overall survival, tumor growth

Introduction

Ovarian cancer is the most lethal and second most common gynecologic malignancy [1]. An estimated 225,500 new cases of ovarian cancer occurred worldwide in 2011 [2, 3]. Also, the ratio of patients with advanced ovarian cancer who have relapses has remained high and fairly constant over the past decade [4]. Furthermore, outcomes of ovarian cancer have changed little over the more than 30 years since the introduction of platinum-based chemotherapy [5]. Early manifestations of malignant ovarian tumors are silent with no evident signs or characteristics, and most patients with ovarian cancer have advanced (stage III or IV) disease at diagnosis; furthermore, about 70% of cases are serous ovarian carcinomas (SOCs) [5, 6]. Thus, identification of both a marker for SOC that can be used for prognosis and targeted therapy and of the relationship between the marker and the effect of chemotherapy with clinic available information is urgently needed.

Using cDNA microarray analysis, we previously detected a profound proinflammatory secretory phenotype induced by oncogenic H-ras^{V12} as well as K-ras^{V12} in the immortalized human ovarian epithelial cell lines T29 and T80 [7]. Expression of vascular cell adhesion molecule 1 (VCAM1) was up-regulated in these cells. The VCAM1 gene is a member of the Ig superfamily [8] and encodes a cell surface sialoglycoprotein expressed in cytokine-activated endothelial

cells [9]. However, VCAM1 protein mediates leukocyte-endothelial cell adhesion and signal transduction [9]. This type I membrane protein not only may play a key role in the development of atherosclerosis [10] and rheumatoid arthritis [11] but also exhibits an important role in the progression of human tumors. Increased serum VCAM1 expression has been found to be related to progression of several cancers [12], including colorectal carcinoma [9, 13, 14], lung cancer [15], bladder carcinoma [16], Hodgkin disease [17], non-Hodgkin lymphoma [18], and others [19]. Also, high VCAM1 expression levels in human tumors such as ovarian cancer [20], esophageal squamous cell carcinoma [21], renal cell carcinoma [22], and breast cancer [23] are of clinicopathologic and prognostic significance. However, the clinical signigicance and role of high VCAM1 expression in ovarian tumor has not been examined.

In this paper, we examined the relationship between VCAM1 protein expression and clinical characteristics of ovarian cancer in 251 patients who underwent treatment at The University of Texas MD Anderson Cancer Center. We also examined the effect of VCAM1 over expression or silencing in ovarian cancer cells and fibroblasts and tested their effect on tumor's development.

Materials and methods

Human ovarian tumor samples and clinicopathologic data

Tumor samples obtained 251 patients diagnosed with high grade ovarian carcinoma who underwent initial surgery at MD Anderson from March 30, 1987, to February 17, 2009, were fixed in formalin and embedded in paraffin. The ovarian tissue blocks were selected by two gynecologic pathologists (H.J. and J.L.) who reviewed hematoxylin and eosin stained tissue microarray. Use of these tissues and chart reviews were approved by the MD Anderson Institutional Review Board. Follow-up information on the study patients was updated through March 31, 2010, by reviewing medical records and the United States Social Security Index. Tumor sample collection and tissue microarray construction were described previously [24]. OS duration was the interval from the date of first biopsy to the date of death because of disease. Patients who were alive on the last follow-up date were censored from the analysis.

Immunohistochemical staining and analysis

Immunohistochemical staining of tissue samples for VCAM1 (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was carried out using avidin-biotin peroxidase methods as described previously [25]. VCAM1 protein expression was scored by scanning tissue microarray slides using a computerized imaging system (Ariol SL-50; Applied Imaging, USA), and the data were reported automatically to a linked clinical database. This sample tracking system was linked with an Access database (Microsoft Corporation, Redmond, WA, USA) containing demographic, clinicopathologic, and survival data for each patient, allowing for rapid linkage of histologic data and clinical features, including International Federation of Gynecology and Obstetrics (FIGO) stage [26], family history of ovarian cancer, age, relapse status, level of clinical response to debulking surgery, presence of ascites, and chemoresponse.

The cutoff point for the VCAM1 expression score that was statistically significant in terms of OS was set using the X-tile software program (The Rimm Lab at Yale University; http://www.tissuearray.org/rimmlab) as described previously [27]. Next, the degree of staining for VCAM1 was quantified using a two-level grading system: staining scores were defined as low expression for samples with less than 30% VCAM1-positive tumor cells, whereas scores were defined as high expression for those with at least 30% VCAM1-positive tumor cells.

Cell lines

The HSOC cell lines OVCA433, OVCA429, and SKOV3 [25] were maintained in Eagle's minimum essential medium (Lonza, Walkersville, MD, USA) with 10% fetal bovine serum. The epithelial cell line T29, malignant ovarian epithelial cell line T29H [7, 28], and ovarian fibroblast line NOF151p53ihT (immortalized NOF151 cells infected with a retrovirus expressing p53 small interfering RNA [siRNA] and a human telomerase catalytic subunit) were described in previous studies [7, 24, 28].

Induction of VCAM1 overexpression using a retrovirus-mediated infection

Total RNA was isolated from 1 x 10^6 T29H cells, which were found to have high levels of VCAM1

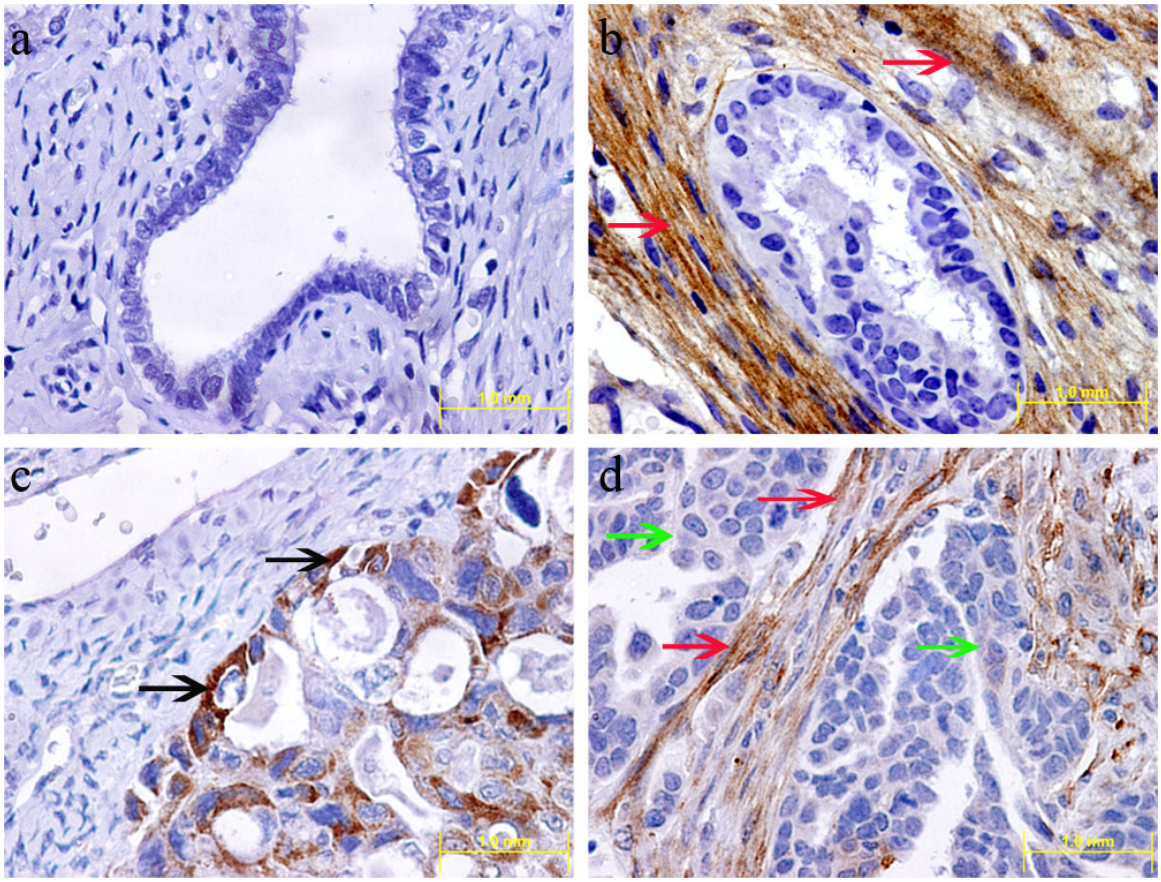

Figure 1. Representative patterns of VCAM1 immunoreactivity in normal fallopian tube, ovarian serous cystadenoma, and ovarian carcinoma tissue samples. A. VCAM1-negative staining in normal fallopian tube tissue. B. Serous cystadenoma tissue with strong VCAM1 positivity in stromal cells (red arrows) and VCAM1-negative staining in epithelial cells. C. Cytoplasmic overexpression of VCAM1 in tumor cells (black arrows) and VCAM1-negative immunohistochemical staining in fibroblasts surrounding the HSOC cells. D. Stromal cells with VCAM1-positive immunostaining (red arrows) were close to the cancer cells. Moreover, the adjacent HSOC cells all exhibited VCAM1-positive immunostaining, although it was quite weak (green arrows). Original magnification, 400x; scale bars, 1 mm.

mRNA expression in a previous study by our group [7]. The primers used to amplify VCAM1 cDNA were 5'-ATATATATATCACGTGACACACAG-GTGGGACACAAA-3' (sense; boldface indicates the *Bam*HI site) and 5'-ATATATATATGT-CGACAACCCAGTGCTCCCTTTGCTTA-3' (antisense; boldface indicates the *Eco*RI site). OVCA433 and OVCA429 cells were infected with viruses containing VCAM1 cDNA according to previously published protocols [29]. Control cell lines were generated via infection of viruses containing an empty vector using the same protocol.

Generation and retroviral delivery of siRNAs against VCAM1 mRNA

SiRNAs against VCAM1 mRNA and a control siRNA were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The protocol for retroviral production and infection to establish the following virus-containing cell lines was carried out as we described previously [30]: T29H-scrambled siRNA (scr), T29H-VCAM1i, NOF151p53ihT-scr, and NOF151p53ihT-VCAM1i.

Western blot analysis of VCAM1 protein expression

Cells that overexpressed VCAM1 protein (OVCA433-VCAM1 and OVCA429-VCAM1), cells in which VCAM1 expression was silenced by VCAM1 siRNA (T29H and NOF151p53ihT), and corresponding control cells that contained empty vectors or scr were maintained in culture flasks. These cells were washed with phosphate-buffered saline (PBS) and lysed sepa-

Table 1. Correlation of VCAM1 expression in tumor cells with clinicopathologic characteristics in HSOC patients

HSOC	n	Low or no expression	High expression	Pearson x^2	*P*
Total	251	201	50		
Stage				4.588	0.101
I-II	12	10 (83.33)	2 (16.67)		
III	175	135 (77.14)	40 (22.86)		
IV	59	53 (89.83)	6 (10.17)		
Unknown	5	3 (60.00)	2 (40.00)		
Response to treatment				7.375	0.025*
Complete	124	107 (86.29)	17 (13.71)		
Partial	77	56 (72.73)	21 (27.27)		
None	30	21 (70.00)	9 (30.00)		
Unknown	20	17 (85.00)	3 (15.00)		
Ascites				3.834	0.050
Yes	187	149 (76.68)	38 (20.32)		
No	33	31 (93.93)	2 (6.06)		
Unknown	31	21 (67.74)	10 (32.26)		
Age at diagnosis				6.981	0.008*
<60 years	112	98 (87.50)	14 (12.50)		
≥60 years	139	103 (74.10)	36 (25.90)		
Family history of ovarian cancer				1.263	0.261
Yes	140	115 (82.14)	25 (17.86)		
No	101	77 (76.24)	24 (23.76)		
Unknown	10	9 (90.00)	1 (10.00)		
CA125 level				1.057	0.304
<600	68	59 (86.76)	9 (13.24)		
≥600	126	102 (80.95)	24 (19.05)		
Unknown	57	40 (70.18)	17 (29.82)		

*$P < 0.05$.

rately with RIPA lysis buffer (Santa Cruz Biotechnology) on ice. The protein concentrations in the lysates were then measured using a protein assay kit (Bio-Rad, Hercules, CA, USA). Equal amounts of total protein (30 μg) were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The membranes were probed with VCAM1 (1:100 dilution). β-actin (Sigma-Aldrich, St. Louis, MO, USA) was used as a loading control. The immune reaction was detected using an ECL WB substrate (Pierce, Rockford, IL, USA) according to the manufacturer's instructions.

Cell transwell migration assays

For analysis of cell colony-forming ability in *vitro*, 5000 T29H, T29H/VCAM1i, OVCA433, OVCA433-VCAM1, OVCA429, OVCA429-VCAM1, NOF151p53ihT, NOF151p53ihT/VCAM1i, and corresponding vector or scr control cells each in 200 μl of medium were added to each well in an 18-well plate (three wells per cell line). Cells were incubated at 37°C in an atmosphere of 5% CO_2 for 72 h. After being washed with PBST, the cells in the plate were stained with crystal violet solution.

A 24-well two-chamber plate with a high-throughput screening multiwell insert (BD Biosciences, San Jose, CA, USA) and an 8-μm (pore size) polycarbonate filter between the chambers was used for a cell migration assay performed as we described previously [29]. T29H, T29H-VCAM1i, OVCA433, OVCA433-VCAM1, OVCA429, OVCA429-VCAM1, NOF151p53ihT, NOF151p53ihT-VCAM1i, and corresponding vector (OVCA433-vector or

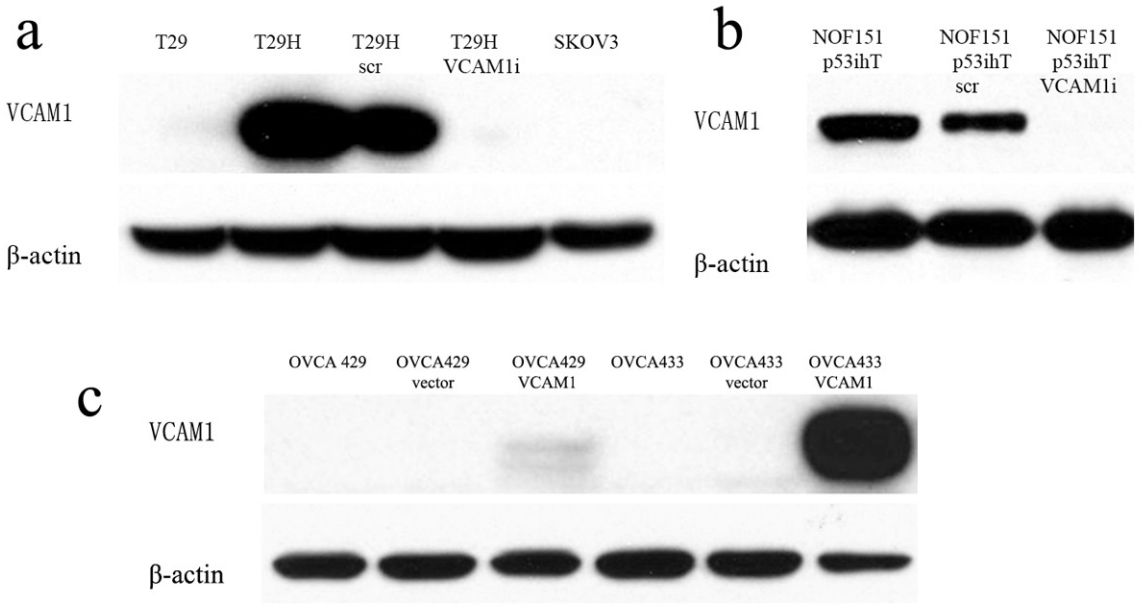

Figure 2. Western blot analysis of VCAM1 protein expression in ovarian cancer cells. VCAM1-silenced cells (T29H-VCAM1 and NOF151p53ihT-VCAM1i) and their corresponding control cells (T29H, T29H-scr, NOF151p53ihT, and NOF151p53ihT-scr), VCAM1-overexpressing cells (OVCA429-VCAM1 and OVCA433-VCAM1) and their corresponding control cells (OVCA429, OVCA429-vector, OVCA433, and OVCA433-vector), and SKOV3 cells were examined. Each lane contained 50 μg of cell lysate protein.

OVCA429-vector) or scr control (T29H-scr or NOF151p53ihT-scr) cells (2.5 x 10^4) were added to each upper chamber toward a lower reservoir containing a medium. Also, 2.5 x 10^4 OVCA433-VCAM1, OVCA433-vector, or OVCA433 cells were separately cultured in the upper transwell chamber with 25,000 NOF151p53ihT cells in the bottom chamber. Furthermore, 2.5 x 10^4 NOF151p53ihT, NOF151p53ihT-scr, or NOF151p53ihT/VCAM1i cells were placed in the lower chamber, whereas 2.5 x 10^4 SKOV3 cells were placed in the upper chamber. All of the cells in the upper chamber were allowed to migrate at 37°C overnight. The migrated cells in the membrane were stained with crystal violet solution after being washed with PBST.

Xenograft ovarian tumors in nude mice

Animal assays were performed according to a protocol approved by the MD Anderson institutional committee for animal experiments. BALB/c athymic nude mice were kept in a pathogen-free environment. Equal numbers of cells (5 x 10^6 T29H-scr or T29H-VCAM1i cells and 2.5 x 10^6 OVCA433-VCAM cells added to 2.5 x 10^6 NOF151p53ihT-scr or NOF151p53ihT-VCAM1i cells) resuspended in 0.15 ml of PBS were injected subcutaneously into 4- to 6-week-old mice (Frederick National Laboratory for Cancer Research, Frederick, MD, USA). The date on which the first grossly visible tumor appeared after injection was recorded for each mouse, and the tumor sizes were measured every week. When a mouse's largest tumor reached 2 cm in diameter, it was killed via exposure to 5% CO_2. Tumor tissue samples then were collected and fixed in formalin, embedded in paraffin, sectioned, and subjected to routine histologic examination by investigators who were blinded to tumor status.

Statistical analysis

The relationship between VCAM1 expression in tumor and FIGO stage, age at diagnosis, family history of cancer, relapse status, clinical response to treat with surgery and drugs, presence of ascites, and other factors were analyzed using the x^2 test or the Pearson correlation coefficient as appropriate. Univariate and multivariate analysis was carried out using the Cox regression method. For all analyses, a *P* value less than 0.05 was regarded as statistically significant. Data were analyzed using the

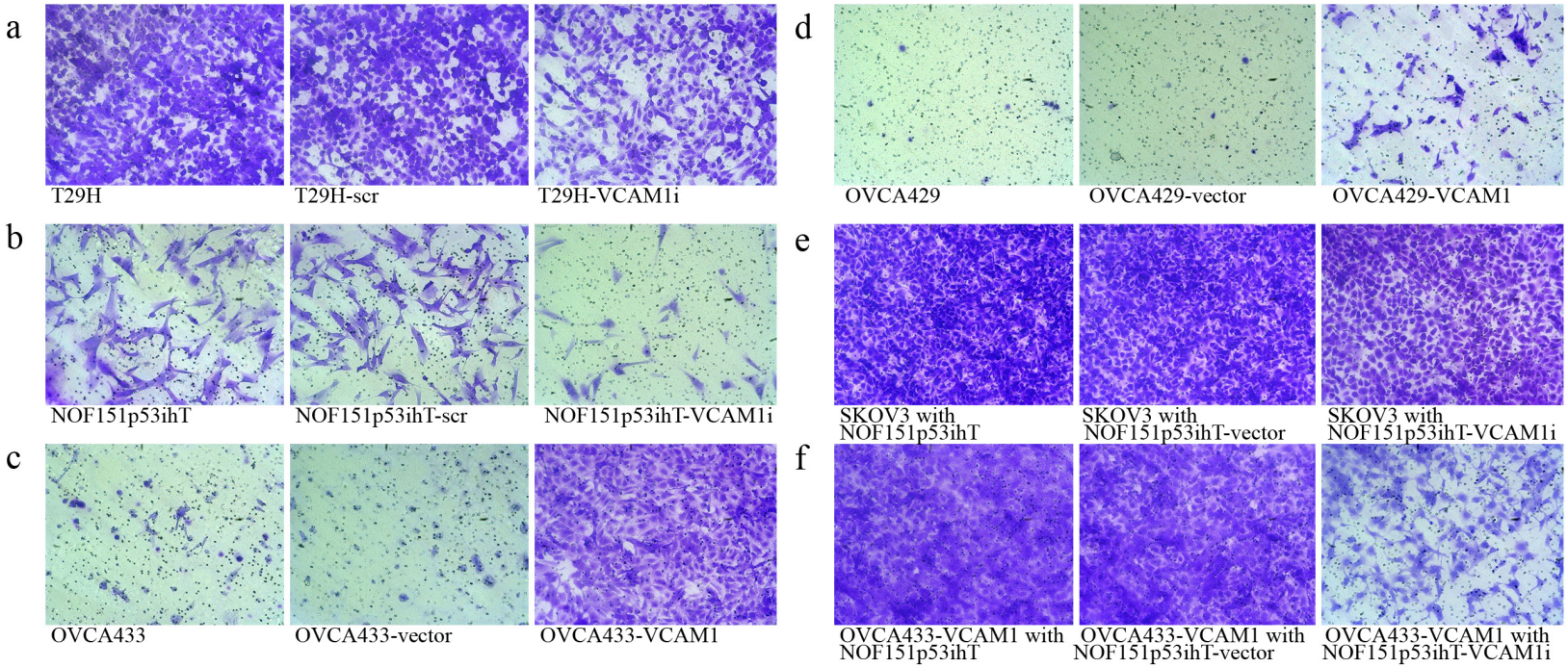

Figure 3. Typical patterns of cell migration in a transwell chamber. A cell-migration ability assay was performed using 24-well 2-chamber plates with a high-throughput screening multiwell insert in medium overnight. The numbers of migrated cells in the cell membranes were observed using crystal violet staining. HSOC cell lines with VCAM1 overexpression (OVCA433-VCAM1 and OVCA429-VCAM1) had greater cell migration ability than did their control cell lines. However, cell lines with silenced VCAM1 (T29H-VCAM1i and NOF151p53ihT-VCAM1i) had lesser migration ability than did their control cell lines. Furthermore, the migration ability of OVCA433-VCAM1 and SKOV3 cells with enhanced secretion of VCAM1 protein from the supernatant of the fibroblast line NOF151p53ihT in the bottom chamber was greater than that of NOF151p53ihT-VCAM1i cells.

STATA (version 9.0; StataCorp, College Station, TX, USA) and SPSS (version 20.0; IBM Corporation, Armonk, NY, USA) software programs. All statistical tests were two-sided.

Results

VCAM1 localization and expression in ovarian tissue samples

We examined the expression of VCAM1 in ovarian tumor and normal ovarian tissue samples using immunohistochemical analysis. Representative immunohistochemical VCAM1 staining patterns are shown in **Figure 1**. VCAM1-positive staining was predominantly localized to the cytoplasm in tumor cells and stromal cells as measured using the computerized imaging system.

Correlation between expression of VCAM1 and clinicopathologic characteristics of HSOC

We analyzed the association of VCAM1 expression with clinicopathologic variables in the study patients. We observed a statistically significant correlation between VCAM1 overexpression (≥30% VCAM1-positive cells) and outcome of these patients (**Table 1**) according to the cutoff point for the VCAM1 expression score as determined using the X-tile software program. High expression of VCAM1 in the ovarian tumor (40%), serous cystadenoma (10%), normal fallopian tube tissue (0%), and normal ovarian tissue (0%) samples differed significantly (x^2 = 9.829, P = 0.02).

Overexpression of VCAM1 in ovarian cancer cells was associated with response to treatment with surgery and drugs (P = 0.025) and age at diagnosis (P = 0.008). In contrast, we found no statistically significant correlations between VCAM1 expression and the following factors: FIGO stage, family history of cancer, presence of ascites, serum CA125 level, and others. All of the data mentioned above are summarized in **Table 1**. However, expression of VCAM1 in fibroblasts was not related to these clinical factors in our patients (Supplemental Table 1).

Association of VCAM1 expression with OS

In Cox regression univariate analysis, low OS rates (at 5 and 15 years after treatment) were related to overexpression of VCAM1 in carcinoma cells (P = 0.029 and P = 0.025, respectively) and stromal cells (P = 0.039 and P = 0.047, respectively), late FIGO stage (P < 0.001 and P < 0.001, respectively), presence of ascites (P = 0.029 and P = 0.014, respectively), and CA125 level in serum (P = 0.011 and P = 0.017, respectively). We included all of these factors in the Cox multivariate analysis, which showed that VCAM1 overexpression in ovarian tumor cells (P = 0.033 and P = 0.049 at 5 and 15 years, respectively) was an independent predictor of OS duration in HSOC patients (**Table 2**).

VCAM1 protein expression and ovarian cancer cell migration

In a previous study, using a cDNA expression array, we showed that expression of VCAM1 mRNA increased 1.1 to 12.7-fold in different RAS-transformed ovarian cell lines over that in vector-transfected control cells [7]. Therefore, in the present study, we first evaluated VCAM1 protein expression in a RAS-transformed ovarian malignant epithelial cell line (T29H) and its corresponding parental cell line (T29), the ovarian fibroblast line NOF151p53ihT, and a panel of HSOC cell lines (OVCA433, OVCA429, and SKOV3) using Western blotting. We observed that VCAM1 protein expression was high in T29H and NOF151p53ihT cells but that VCAM1 protein was not expressed in OVCA433, OVCA429, or SKOV3 cells (**Figure 2**).

To examine the effect of increased expression of VCAM1 protein on cell lines, we performed retroviral infection to introduce a VCAM1 cDNA expression vector into OVCA433 and OVCA429 cells. Also, we established T29H-VCAM1i and NOF151p53ihT-VCAM1i cells with retroviruses carrying siRNA against VCAM1 to silence VCAM1 expression and control cells (with an empty vector or scr). We found that VCAM1 protein expression increased after introducton of VCAM1 cDNA and decreased after infection of retrovirus expressing VCAM1 siRNA in the cells. The levels of expression of VCAM1 protein in these cell lines are shown in **Figure 2**.

We next evaluated the migration ability of cell lines whose expression of VCAM1 protein was higher or lower than that in corresponding control cells containing an empty vector or control siRNA. The cells that overexpressed VCAM1 protein (OVCA433-VCAM1 and OVCA429-

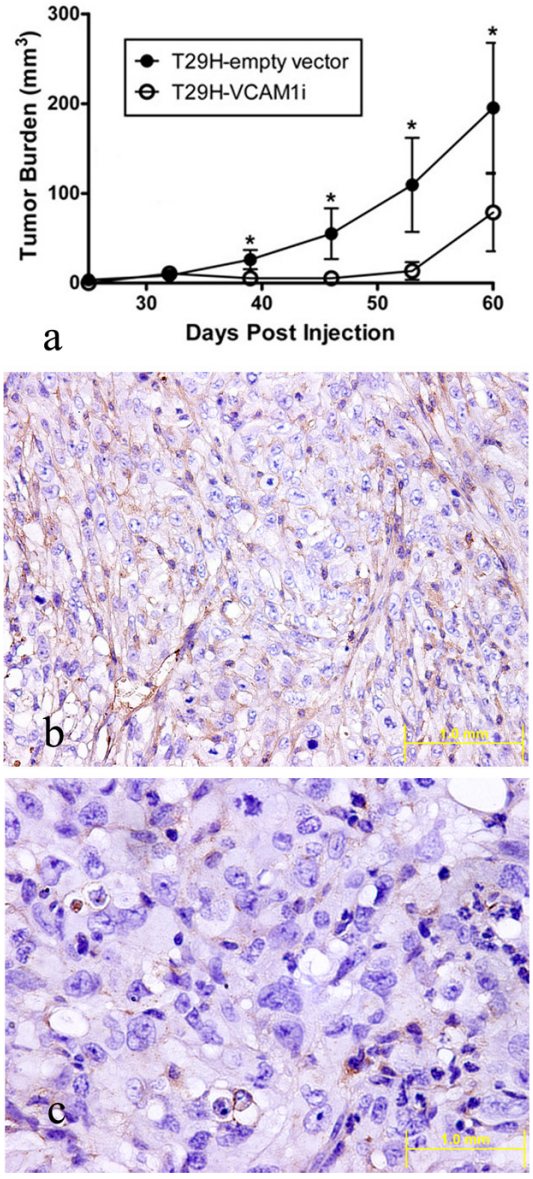

Figure 4. The xenograft ovarian tumor burden in mice and VCAM1 expression in tumor samples obtained from mice. A. Growth of ovarian tumors produced by subcutaneous injection of T29H-VCAM1i cells or vector control cells. Each mouse received injections at two sites to form two tumors per mouse. Error bars, 95% CIs. (B and C) Stronger VCAM1-positive immunostaining and higher cell density in a T29H-scr tumor sample (B) than in a T29H-VCAM1i tumor sample (C).

VCAM1) had greater migration abilities than did the corresponding control cells. In contrast, the cells whose VCAM1 expression was silenced by siRNA (T29H-VCAM1i and NOF151p53ihT-VCAM1i) had lesser cell migration (**Figure 3A-D**) abilities than did the corresponding control cells.

In addition, we observed that SKOV3 cells cultured with NOF151p53ihT-VCAM1i stromal cells in the lower transwell chamber exhibited less migration ability than did SKOV3 cells cultured with NOF151p53ihT or NOF151p53ihT-scr cells (**Figure 3E**). Furthermore, OVCA433-VCAM1 cells had greater migration ability than did the corresponding control cells, which were individually accompanied by NOF151p53ihT cells in the lower transwell chamber (**Figure 3F**).

VCAM1 expression in ovarian cells and ovarian tumorigenesis in vivo in nude mice

To determine the effect of altering VCAM1 expression in tumor cells on ovarian tumor growth, we used injection of ovarian cancer cells in which VCAM1 was overexpressed or its expression was silenced to establish human ovarian cancer xenografts in nude mice. OVCA433-VCAM1 cells and the corresponding control cells, even when injected with NOF151p53ihT or NOF151p53ihT-VCAM1i cells, formed small nodule that rapidly disappeared in 2 weeks in mice.

T29H-scr tumors were obviously larger than T29H-VCAM1i tumors over the 20-week tumor-growth observation period (**Figure 4A**). As demonstrated by immunohistochemical analysis, the degree of VCAM1-positive staining in the cytoplasm of T29H-scr cells (**Figure 4B**) was higher than that in the cytoplasm of T29H-VCAM1i cells (**Figure 4C**). Thus, these data demonstrated that VCAM1 protein is required for RAS-mediated transformation of ovarian epithelial cells.

Discussion

We previously identified VCAM1 as a target gene after introduction of the RAS oncogene in immortalized ovarian epithelial cells in our original study [7]. In the present study, we found that the VCAM1 overexpression occurred not only in ovarian cancer cells but also in stromal cells in ovarian cancers. Because HSOC is the most common subtype of ovarian cancer and has distinct molecular characteristics, including genetic instability and mutations of p53 [31, 32], we selected a cohort of HSOC patients for this study. Our data suggested that VCAM1 protein overexpression in HSOC cells was associated with a poor prognosis. Our clinical data also demonstrated that VCAM1 overexpression

Table 2. Univariate and multivariate analysis of HSOC prognostic factors for OS at 5 and 15 years after the first treatment

Characteristic	Years	Univariate analysis				Multivariate analysis			
		HR	*P*	95% CI		HR	*P*	95% CI	
VCAM1 expression in tumor cells									
High vs low	5	1.594	0.029*	1.049	2.423	1.937	0.033*	1.054	2.424
	15	1.587	0.025*	1.059	2.376	1.825	0.049*	1.002	3.325
VCAM1 expression in stromal cells									
High vs low	5	0.683	0.039*	0.475	0.982	0.698	0.126	0.440	1.107
	15	0.703	0.047*	0.497	0.995	0.734	0.176	0.469	1.148
Stage									
I-II vs III vs IV	5	2.207	0.000*	1.572	3.098	4.434	0.055	0.968	20.354
	15	2.121	0.000*	1.535	2.930	3.375	0.066	0.923	12.334
Family history of ovarian cancer									
Yes vs no	5	1.064	0.707	0.770	1.469				
	15	1.130	0.412	0.843	1.514				
Ascites									
Yes vs no	5	1.957	0.029*	1.073	3.572	1.588	0.230	0.746	3.382
	15	2.031	0.014*	1.156	3.569	1.713	0.147	0.828	3.543
Age (years)									
<60 vs ≥60	5	1.094	0.621	0.766	1.562				
	15	1.178	0.347	0.837	1.660				
Presurgery vs post-surgery	5	0.710	0.125	0.459	1.100				
	15	0.757	0.207	0.491	1.166				
CA125 level									
<600 vs ≥600	5	1.837	0.011*	1.150	2.933	1.474	0.139	0.882	2.463
	15	1.724	0.017*	1.103	2.700	1.448	0.142	0.884	2.371

*$P < 0.05$. HR: hazard ratio, CI: confidence interval.

was linked with advanced age at diagnosis and poor response to surgery and chemotherapy. Specifically, overexpression of VCAM1 not only was common in elderly patients but also was associated with chemoresistance. However, VCAM1 expression in fibroblasts was not significantly related to any clinicopathologic parameters. We also showed that VCAM1 was required for RAS-mediated ovarian tumor growth *in vivo*. Taken together, our clinical and experimental data support that VCAM1 may be a prognostic factor and novel therapeutic target for HSOC.

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Supplemental Table 1. Correlation of VCAM1 expression in ovarian stromal cells with clinicopathologic characteristics in HSOC patients

HSOC	n	Low or no expression	High expression	Pearson x^2	*P*
Total	251	131	120		
Stage				0.3150	0.854
I-II	12	7 (58.33)	5 (41.67)		
III	175	90 (51.43)	85 (48.57)		
IV	59	32 (54.24)	27 (45.76)		
Unknown	5	2 (40.00)	3 (60.00)		
Response to treatment				2.9410	0.230
Complete	124	58 (46.77)	66 (53.23)		
Partial	77	44 (57.14)	33 (42.86)		
None	30	18 (60.00)	12 (40.00)		
Unknown	20	11 (55.00)	9 (45.00)		
Ascites				0.1160	0.734
Yes	187	96 (51.34)	91 (48.66)		
No	33	18 (54.55)	15 (45.45)		
Unknown	31	17 (54.84)	14 (45.16)		
Age at diagnosis				0.0190	0.890
<60 years	112	59 (52.68)	53 (47.32)		
≥60 years	139	72 (51.80)	67 (48.20)		
Family history of ovarian cancer				0.0003	0.987
Yes	140	75 (53.57)	65 (46.43)		
No	101	54 (53.47)	47 (46.53)		
Unknown	10	2 (20.00)	8 (80.00)		
CA125 level()				0.3820	0.537
<600	68	35 (51.47)	33 (48.53)		
≥600	126	59 (46.83)	67 (53.17)		
Unknown	57	37 (64.91)	20 (35.09)		