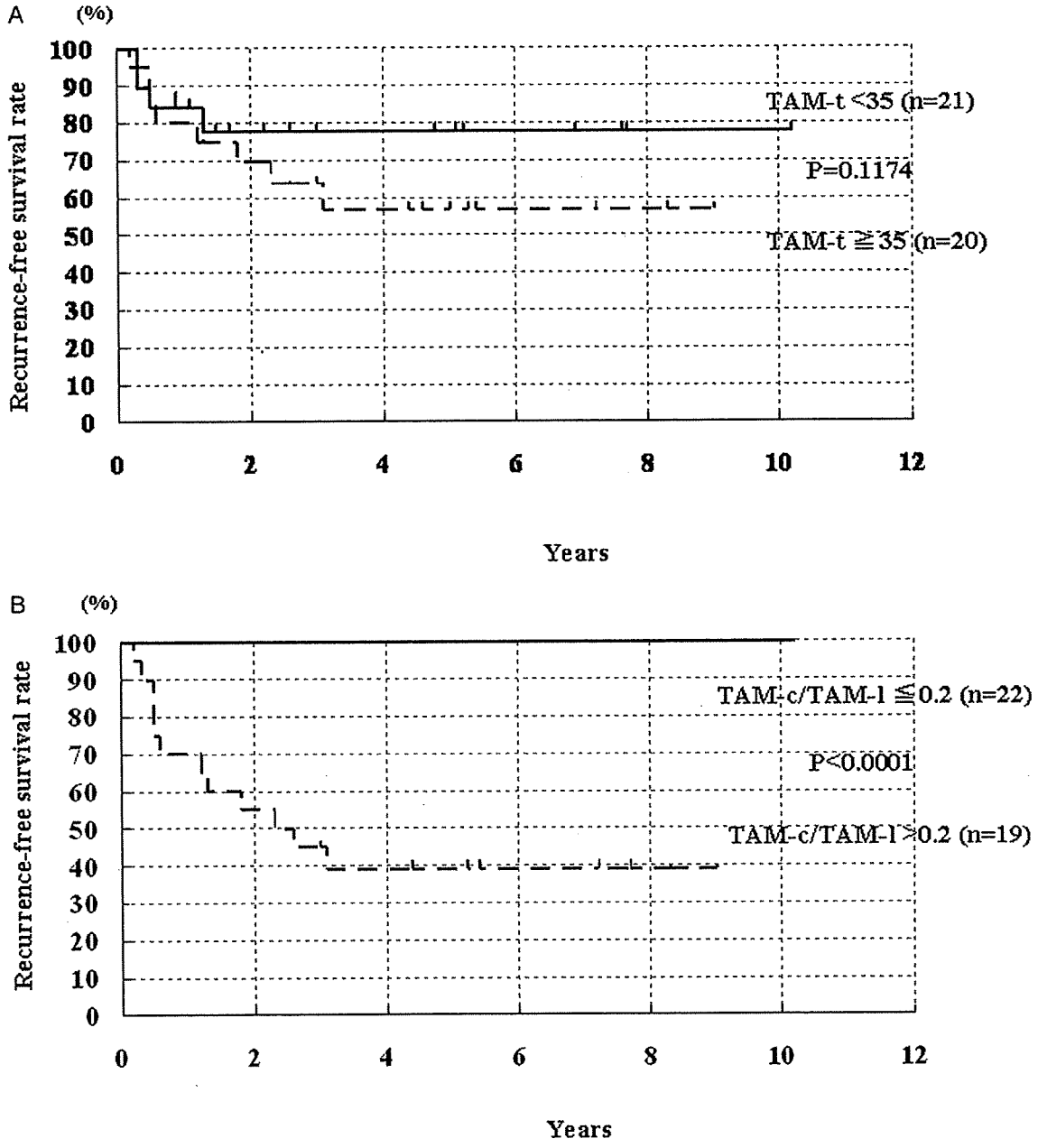

Figure 3. Recurrence-free survival in patients with bladder CIS and high or low TAM count. *A*, tissue. *B*, TAM-c/TAM-l ratio (significantly different, p <0.0001).

Our immunohistochemical studies have shown that TAMs are present in the lamina propria as well as among tumor cells in the epithelial area of bladder CIS. In terms of cell-cell interaction we hypothesized that TAMs among cancer cells may have more important roles than those in the lamina propria. Interestingly our data indicate that TAM infiltration in the cancer area is more important as a prognostic factor than infiltration in the lamina propria. TAMs located in the cancer area may interact directly with cancer cells. However, in this study TAMs were identified by immunohistochemistry using CD68 antibody. There is a possibility that CD68 positive TAMs are composed of 2 or more functionally different components. Sica et al reported that there are M1 and M2 macrophages, and M2 macrophages correspond to TAMs.[13] They also reported that M2 cells are composed of several functionally different macrophages. Some component of M2 may possibly correspond to TAMs in the cancer area and other to TAMs in the lamina propria.

Table 3. *Prognostic factors on Cox multivariate analysis*

Prognostic Factor	HR	95% CI	p Value
Sex (M vs F)	0.8677	0.094–6.913	0.8677
Age (continuous variable)	1.084	1.009–1.164	0.0641
TAM:			
No. total area (35 or greater vs less than 35)	1.040	0.988–1.095	0.2125
No. Ca (4 or greater vs less than 4)	1.737	1.476–5.034	0.0012
No. lamina propria (24 or greater vs less than 24)	1.009	0.918–1.1.09	0.8738
Ratio (0.2 or greater vs less than 0.2)	12.685	3.068–52.457	0.0032

TAMs have been reported to have various functions, such as the inhibition of cancer growth, destruction of cancer cells and promotion of tumor progression (the macrophage balance hypothesis).[11] Accumulating evidence suggests that TAMs are part of the inflammatory circuit that promotes tumor progression.[11–14] The presence of TAMs positively correlates with increased vascularity and metastasis, and with decreased relapse-free and overall survival in breast cancer.[15] Hanada et al found that an increased TAM count in bladder cancer correlates with progression and poor prognosis.[17] Moreover, another 2 studies showed that the expression of endothelial Per-Arnt-Sim domain protein 1 (EPAS1) by TAMs is a good prognostic factor.[18] These reports describe that TAM infiltration leads to bladder cancer progression via neovascularization.

We investigated the recurrence of bladder CIS but not of papillary or solid bladder tumors after BCG instillation. Therefore, the scope of our study differs from that of previous studies, although our data support the finding that TAMs positively influence tumor growth and progression. In bladder CIS microvessels can only be observed in the lamina propria but not in the cancer cell layer. Therefore, in cases of bladder CIS TAMs among bladder cancer cells may support the growth of cancer cells with some growth factors or cytokines via cell-cell interactions, or they may protect cancer cells from attack by the immune responsive cells induced by BCG.

Despite its clinical benefit to our knowledge the mechanism underlying the antitumor activity of intravesical BCG instillation has not been clarified. However, it has been reported that intravesical BCG instillation is associated with many immunological events.[13,19,20] Intravesical BCG initially depends on the attachment of BCG to bladder tumor cells via fibronectin.[20] BCG attached to the bladder tumor cell is internalized in tumor cells and macrophages in the lamina propria.[20] Macrophages are activated and they release cytokines, tumor necrosis factor-α, IL-12 and interferon-γ.[19] Indeed, we observed many macrophages in the lamina propria but the correlation between TAM infiltration in the lamina propria and recurrence-free survival was not significant. It is notable that TAM infiltration in the cancer area correlated well with recurrence-free survival.

To date only a few prognostic markers that predict the response to BCG instillation therapy have been reported. Specific cytokines such as IL-2 and IL-8 in urine have been reported to be prognostic factors for tumor recurrence after BCG instillation.[7–10] Moreover, these factors can only be observed after BCG therapy. Because BCG instillation causes severe adverse effects such as cystitis or a contracted bladder in some patients, it would be useful to discover good markers that distinguish responders and nonresponders to BCG instillation before therapy. Our multivariate analysis indicates that TAM infiltration in the cancer area is an independent prognostic indicator of recurrence-free survival. Using TAM infiltration in the cancer area would allow the prognosis of bladder CIS treated with intravesical BCG to be predicted before the initiation of therapy. TAM immunostaining can be used to potentially discern whether intravesical BCG therapy is an appropriate choice in patients with a low level of TAM infiltration in the cancer area because the risk of tumor recurrence after therapy is low. In conclusion, as determined by immunohistochemistry, TAM infiltration could be used as an indicator of the response of bladder CIS to intravesical BCG instillation and for selecting the primary treatment modality.

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Vascular endothelial growth factor receptor 1 expression in pelvic lymph nodes predicts the risk of cancer progression after radical prostatectomy

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Recent studies suggest that vascular endothelial growth factor receptor (VEGFR) 1-positive hematopoietic progenitor cells precede the arrival of tumor cells and form clusters that may portend sites of future metastatic disease. The aim of the present study was to clarify whether VEGFR1 expression in pelvic lymph nodes predicts the risk of prostate cancer progression after radical prostatectomy. VEGFR1 expression in pelvic lymph nodes was examined by immunohistochemistry in 95 patients who underwent radical prostatectomy for prostate cancer. A cluster of VEGFR1-positive cells was considered positive. Expression of VEGFR1 in pelvic lymph nodes and biochemical recurrence after radical prostatectomy were examined by univariate survival analysis and multivariate Cox proportional hazards regression analysis. Thirty-seven of 79 lymph node-negative patients (46.8%) were found to have VEGFR1-positive cells in their pelvic lymph nodes, whereas 16 of 16 lymph node metastasis-positive patients (100%) had VEGFR1 clusters. There was a significant correlation between pathological stage and VEGFR1 staining ($P = 0.002$). Univariate analysis showed that pathological stage ≥pT3 and VEGFR1 expression in pelvic lymph nodes were each significantly associated with biochemical recurrence after radical prostatectomy. Multivariate analysis showed VEGFR1 expression to be an independent predictor of biochemical recurrence after radical prostatectomy (risk ratio = 5.715, $P = 0.010$), as was preoperative prostate-specific antigen (PSA) level ≥10 ng/mL. Although larger validation studies are required, our results suggest that VEGFR1 expression in pelvic lymph nodes predicts the risk of biochemical PSA recurrence after radical prostatectomy. (*Cancer Sci* 2009; 100: 1047–1050)

Prostate cancer is the most common cancer and the second leading cause of cancer-related death in men over 40 years old in the USA.[1] The major cause of death is metastasis that is resistant to therapy. Approximately 25% of patients with clinically localized prostate cancer suffer the progression of disease after radical prostatectomy.[2] Of the patients undergoing radical prostatectomy, 20–50% will suffer biochemical relapse.[3] Adjuvant radiotherapy and hormonal therapy can be administered after radical prostatectomy for patients at increased risk.[4] However, some patients could suffer possible side effects and the associated expense of unnecessary adjuvant therapy. The development of markers that can predict prognosis after prostatectomy is crucial to identify patients who can benefit from further therapy.

The microenvironment of tumor-draining lymph nodes contains numerous biologically active molecules, including cytokines and chemokines, that are produced locally and imported into the lymph node from the area of the tumor.[5] The sentinel lymph nodes are known to be the first lymphoid organ to respond to these stimulations from tumors. Therefore, changes in the sentinel lymph nodes may precede metastasis or recurrence of cancers.

Recently, it was shown that bone marrow-derived hematopoietic progenitor cells that express vascular endothelial growth factor receptor (VEGFR) 1 home in on tumor-specific premetastatic sites and form cellular clusters, termed 'premetastatic niches', before the arrival of tumor cells. The number of VEGFR1-positive cells also increases in the peripheral blood of patients with metastatic gastric cancer.[6]

These findings led us to hypothesize that pelvic lymph nodes may contain VEGFR1-positive cells before metastasis or prostate-specific antigen (PSA) failure occur. In this study, we immunohistochemically examined pelvic lymph nodes dissected at radical prostatectomy for the presence of VEGFR1-positive cells.

Materials and Methods

Study population and tissue samples. Immunohistochemical examination was carried out retrospectively on lymph node specimens that were taken from patients with prostate cancer ($n = 95$) who underwent pelvic lymph node dissection and radical prostatectomy. The procedure was carried out at Osaka University Hospital or Osaka Rosai Hospital between July 1999 and December 2006. Serum concentrations of PSA were determined preoperatively. The specimens were fixed in formaldehyde, embedded in paraffin, and cut into 5 μm-thick sections. All patients provided written informed consent to participate in the study.

Vascular endothelial growth factor receptor 1 immunohistochemistry. For antigen retrieval, slides were incubated in citrate-buffered saline (30 min at 98°C). The primary antibody was a rabbit polyclonal antibody generated against recombinant human VEGFR1 (C17; Santa Cruz Biotechnology, Santa Cruz, CA, USA). After the endogenous peroxidase activity was blocked with 3% H_2O_2, sections were incubated for 1 h at 37°C with primary antibody at a dilution of 1:200. Antibody was detected by the streptavidin–biotin–peroxidase method with diaminobenzidine by means of an EnVision kit (Dako, Kyoto, Japan). The sections were counterstained with 10% hematoxylin, dehydrated, and mounted. Negative controls were prepared with the use of normal rabbit IgG (Vector Laboratories, Burlingame, CA, USA) as the primary antibody. A positive control was prepared with a human angiosarcoma. Microscopic analysis was carried out by two independent investigators (K.F. and M.N.), and a cluster of VEGFR1-positive cells in the follicle was considered positive.

Statistical analysis. The statistical significance of the association between VEGFR1 expression and clinicopathological parameters was assessed with the χ^2-test for trends or Fisher's exact test.

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Table 1. Characteristics of 79 patients with prostate cancer and results of vascular endothelial growth factor receptor (VEGFR) 1 staining in lymph nodes

Characteristic	All patients	VEGFR1 negative	VEGFR1 positive	*P*-value
Patients (*n*)	79	42	37	
Median age (years) (range)	67 (51–76)	67 (57–75)	67 (51–76)	0.45
Median preoperative PSA (ng/mL) (range)	8.3 (3.3–60.4)	8.0 (3.3–44.0)	9.3 (3.7–60.4)	0.98
Median no. lymph nodes (range)	5 (1–15)	4 (1–15)	6 (2–11)	0.13
Pathological stage				0.002
pT2	49	33	16	0.001†
pT3	30	9	21	
pT3a	20	6	14	
pT3b	10	3	7	
Gleason score				
≤6	25	15	10	0.06
7	36	22	14	
≥8	18	5	13	
Surgical margins				
Negative	67	39	28	0.03
Positive	12	3	9	

†pT2 versus pT3. PSA, prostate-specific antigen.

Biochemical (PSA) progression after radical prostatectomy was defined as PSA >0.2 ng/mL. For 66 of 67 patients with negative surgical margin and without metastasis, the expression of VEGFR1 in pelvic lymph nodes and biochemical progression after radical prostatectomy were examined by univariate survival analysis with Kaplan–Meier survival curves with the log-rank test and multivariate Cox proportional hazards regression. One of these 67 patients was excluded from this analysis because postprostatectomy information could not be obtained. Statistical significance was defined as a *P*-value <0.05.

Results

VEGFR1 immunostaining in lymph nodes. First, pelvic lymph nodes with lymph node metastasis from 16 patients with prostate cancer were examined for immunostaining of VEGFR1. Immunohistochemical staining of lymph nodes showed that most VEGFR1-positive cells were found in venules or lymphatic sinuses and some cells in the follicles. A cluster of VEGFR1-positive cells was noticed in the follicles of all 16 patients with lymph node metastasis (Fig. 1).

Next, we immunohistochemically examined pelvic lymph nodes from 79 prostate cancer patients without lymph node metastasis for clusters of VEGFR1-positive cells in follicles of pelvic lymph nodes. The clinicopathological characteristics are summarized in Table 1. Of the 79 patients without lymph node metastasis, 49 patients were classified as pathological stage T2 (pT2), 20 as pT3a, and 10 as pT3b. Of the 79 patients without lymph node metastasis, 25 patients had a total Gleason score of 6 or less, 36 patients had a Gleason score of 7, and 18 patients had a Gleason score ≥8. VEGFR1-positive cells in pelvic lymph nodes were found in 37 of 79 lymph node-negative patients (46.8%), whereas 16 of 16 lymph node metastasis-positive patients (100%) had VEGFR1-positive cells, and the difference was statistically significant ($P < 0.01$). The median ratio of the number of VEGFR1-positive lymph nodes to the total number of dissected lymph nodes was 0.53 (range 0.18 to 1.0) in patients with lymph node metastasis and 0.25 (ranged 0.1 to 1.0) in patients without lymph node metastasis. The preoperative PSA values of patients with VEGFR1-positive lymph nodes were not different from those of negative patients ($P = 0.98$) (Table 1). The percentage of pT3 patients positive for VEGFR1 (70.0%, 21 of 30 patients) was higher than that of pT2 patients (32.7%, 16 of 49 patients), and this difference was statistically significant ($P = 0.001$). Ten of 25 patients (40.0%) with a Gleason score of 6 or less, 14 of 36 patients (38.3%) with a Gleason score of 7, and 13 of 18 patients (72.0%) with Gleason score ≥8 were positive for VEGFR1, but this tendency was not statistically significant ($P = 0.06$).

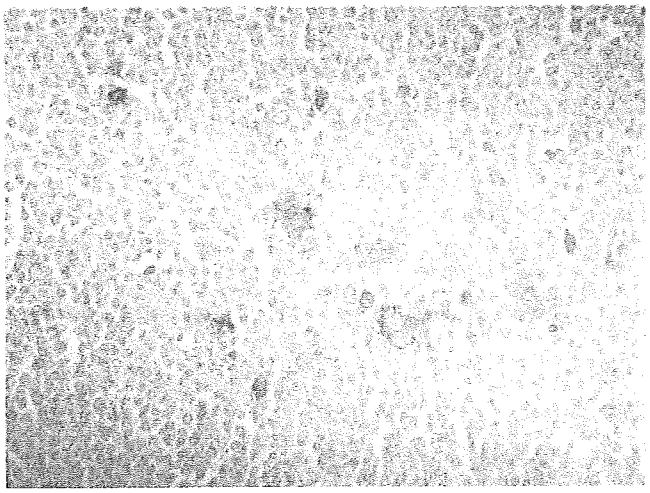

Fig. 1. Immunohistochemical analysis for vascular endothelial growth factor receptor (VEGFR) 1 in a pelvic lymph node. Clusters of VEGFR1-positive cells were identified in pelvic lymph nodes from patients with prostate cancer who underwent radical prostatectomy.

Association of VEGFR1 with biochemical recurrence. We analyzed the correlation between VEGFR1 and biochemical PSA recurrence in a subset of patients with negative surgical margin. Sixty-six of 79 patients (83.5%) without lymph node metastasis had negative surgical margin. The median follow-up period of these 66 patients was 24 months (range, 0–50 months). PSA recurrence after radical prostatectomy was observed in 15 of these 66 patients (22.7%). Univariate analysis showed that VEGFR1 expression in pelvic lymph nodes (Fig. 2) and pathological stage pT3 were significantly associated with biochemical recurrence after radical prostatectomy (Table 2). On multivariate analysis, a preoperative PSA level more than 10 ng/mL and VEGFR1 expression were independent predictors of biochemical recurrence after radical prostatectomy (Table 2).

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Table 2. Univariate and Multivariate analysis of clinical and pathological features and vascular endothelial growth factor receptor (VEGFR) 1 staining in lymph nodes for the prediction of prostate-specific antigen (PSA) progression in 66 patients

Feature	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	RR	95% CI	*P*-value
Age (<65 years)	2.656	0.9870–8.1898	0.0538	3.164	0.9518–10.5162	0.0602
Preoperative PSA (≥10 ng/mL)	1.898	0.6978–5.9694	0.2161	4.023	1.1623–13.9266	0.0280
Gleason score (≥7)	5.340	0.9379–9.4476	0.0696	2.830	0.3527–22.7013	0.3275
Pathological stage (≥pT3)	3.532	1.5907–21.2906	0.0092	2.862	0.8238–9.9417	0.0980
VEGFR1 positive	7.748	2.7831–24.3130	0.0002	5.715	1.5064–21.6791	0.0104

CI, confidence interval; HR, hazard ratio; RR, relative risk.

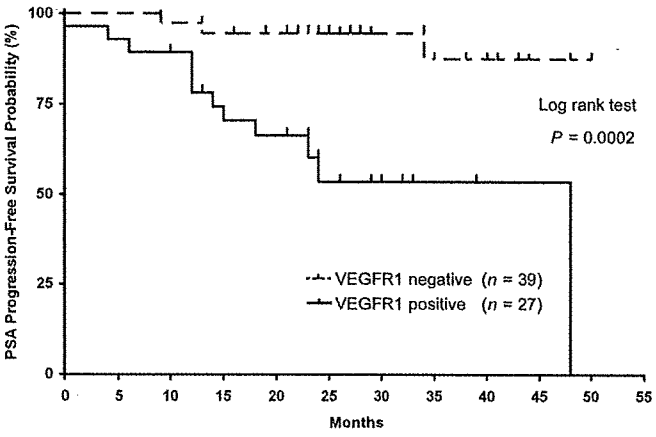

Fig. 2. Kaplan–Meier recurrence curves of biochemical progression-free probability for vascular endothelial growth factor receptor (VEGFR) 1-positive cases and VEGFR1-negative cases in 66 patients without lymph node metastasis and with negative surgical margin. PSA, prostate-specific antigen.

Discussion

In the present study, we showed that clusters of VEGFR1-positive cells in lymph nodes correlated with biochemical recurrence of prostate cancer after radical prostatectomy. There have been no reports to our knowledge that have studied the clinical significance of VEGFR1 expression in the lymph nodes of patients with prostate cancer.

The vascular endothelial growth factor (*VEGF*) gene family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF) and binds to the three receptor tyrosine kinases VEGFR1 (Flt-1), VEGFR2 (Flk-1), and VEGFR3 (Flt-4).[7] VEGF-A binds to VEGFR1 and VEGFR2, and PlGF and VEGF-B bind only to VEGFR1.[8] VEGF-C and VEGF-D bind to VEGFR2 and VEGFR3. VEGFR1 and VEGFR2 are highly expressed in vascular endothelial cells and function mainly in angiogenesis.[8] VEGF-C and VEGF-D can induce lymphangiogenesis by activating VEGFR3, which is expressed on the lymphatic endothelial cells.[9] VEGF-C and VEGF-D induce lymphangiogenesis at the primary tumor sites and promote metastasis.[10,11] The expansion of lymphatic networks is also induced within lymph nodes even before the onset of metastasis by VEGF-C and promotes tumor metastasis to the lymph nodes.[12]

Prostate cancer expresses VEGF-A, VEGF-B, VEGF-C, and VEGF-D.[13–15] In prostate cancer, VEGF-A expression has been correlated with tumor progression,[13] whereas VEGF-C and VEGF-D expression has been associated with lymph node metastasis.[14,15] Prostate cancer cell lines (PC3, DU145, and LNCaP) do not express VEGFR1,[16] but immunohistochemical analysis has shown the expression of VEGFR1 in primary prostate cancer.[17,18] VEGFR3 is also expressed by prostate cancer as well as by lymphatic endothelial cells, and its expression has been correlated with lymph node metastasis.[15,19]

Interestingly, recent studies suggest the correlation of VEGFR1 or VEGF-A with lymphangiogenesis. Hirakawa *et al.* reported that VEGF-A also induces lymph node lymphangiogenesis before metastasizing.[20] PlGF selectively binds VEGFR1, but anti-PlGF inhibits tumor lymphangiogenesis.[21] VEGF-A recruits monocytes or macrophages to inflamed mouse cornea and stimulates lymphangiogenesis by release of VEGF-C and VEGF-D from macrophages.[22] VEGFR1 signaling promotes lymphangiogenesis indirectly by increasing bone marrow-derived macrophage recruitment.[23] Therefore, it is plausible that cytokines or chemokines draining into lymphatic vessels and sentinel lymph nodes from primary tumors attract VEGFR1-positive cells to lymph nodes and that these VEGFR1-positive cells induce intralymph node lymphangiogenesis and promote metastasis and biochemical failure.

We found VEGFR1-positive cells in follicles, with which B cells are mainly associated, in the outer cortex.[5] The paracortical area of lymph nodes includes antigen-presenting dendritic cells transported from the tumor environments and T cells. The function of VEGFR1-positive cells in follicles remains to be determined.

A limitation of our study is that we did not identify the origin of VEGFR1-positive cells in lymph nodes. Hematopoietic stem cells and progenitors express VEGFR1 and c-kit and home in on metastatic niches or tumor endothelial cells.[24,25] However, the VEGFR1-positive cells in the lymph nodes in the present study were negative for c-kit by our immunohistochemical analysis (data not shown). Monocytes are known to express VEGFR1, to be associated with lymphangiogenesis at the lymph node, and to promote metastasis. Immunohistochemistry with anti-CD68 antibody showed that many CD68-positive cells were in lymphatic sinuses, and some cells in the follicle, which looked same as VEGFR1-positive cells in lymph nodes (data not shown). The possible source of VEGFR1-positive cells may be monocytic lineage. Further study such as double-label fluorescence immunohistochemistry will be required to elucidate the characteristics of VEGFR1-positive cells.

In conclusion, our results suggest that clusters of VEGFR1-positive cells in pelvic lymph nodes predict the risk of PSA recurrence after radical prostatectomy. Further studies will be necessary to validate this initial observation.

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Decreased immunostaining for macrophage scavenger receptor is associated with poor prognosis of prostate cancer

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Study Type – Aetiology (case series)
Level of Evidence 4

OBJECTIVE

The aim of this study is to evaluate the expression of the macrophage scavenger receptor (MSR) in prostate needle biopsy specimens as a possible prognostic factor for prostate cancer. As MSR reportedly has a role in recognizing foreign pathogenic substances, MSR-positive inflammatory cells are often detected in solid tumours, and there is a correlation between the relative risk of prostate cancer and polymorphism of the MSR gene.

PATIENTS AND METHODS

MSR was evaluated by immunostaining in needle biopsies of the prostate from 135 patients who were confirmed to have prostate cancer. Among these men, 70 were treated by radical prostatectomy or by radiotherapy as definitive therapy; the other 65 were treated by hormonal therapy because of advanced disease or age. Needle-biopsy specimens were sectioned at 5 μm and immunostained with a monoclonal antibody against MSR. Six microscopic (×400) fields around the cancer foci were selected in each case for analysis.

RESULTS

The median number of MSR-positive cells (MSR count) in each case was 24. There was an inverse correlation between the MSR count and Gleason score and clinical stage. The MSR count was lower in patients with biochemical (prostate-specific antigen, PSA) failure than that in those with no PSA failure ($P < 0.001$). In all patients, the recurrence-free survival (RFS) rate was significantly higher in those with a high MSR count (≥24) than that in those with low MSR count (<24, $P < 0.001$). Moreover, for patients treated by definitive or hormonal therapy, the RFS rates in those with a higher MSR count were higher than in those with a lower MSR count ($P < 0.001$ and 0.014, respectively). Cox multivariate analysis showed that the MSR count was a prognostic factor for prostate cancer in addition to extraprostatic extension and Gleason score ($P = 0.002$, 0.038 and 0.011, respectively).

CONCLUSION

The results of immunostaining of MSR in needle-biopsy specimens is a prognostic factor for prostate cancer.

KEYWORDS

macrophage scavenger receptor, prostate cancer, needle biopsy, prognosis, immunohistochemical analysis

INTRODUCTION

Prostate cancer is one of the most common cancers and is the second leading cause of cancer-related deaths in men in the USA [1,2]. The incidence of prostate cancer has been gradually increasing in Japan recent years. Although most patients currently are diagnosed with clinically localized disease, 30–40% fail to respond to local definitive therapy (radiation or surgery) within 10 years, as shown by an increase in the PSA level [3,4]. Previous studies has shown that clinical stage, tumour grade and PSA level are prognostic factors for prostate cancer [5,6]. However, there are only a few reports of prognostic factors reflecting an immune response by the host against cancer and characteristics of prostate cancer itself [7].

The macrophage scavenger receptor 1 gene (*MSR1*) is located on the short arm of chromosome 8p22–23, and MSR1 protein functions in several processes which are related to prostate carcinogenesis [8–11]. Moreover, mutations in *MSR1* have been reported to be associated with the risk of prostate cancer [12,13]. MSR1 is a homotrimeric integral membrane protein that acts as a receptor for multiple polyanionic ligands, including bacteria [8]. Although MSR is located predominantly in macrophages, its expression has been reported in vascular endothelial cells, smooth muscle cells, fibroblasts, and prostate cancer [14–16]. A decrease in the number of MSR-positive cells indicates tumour progression, as shown by clinical and pathological correlations in prostatic surgical specimens [16]. However, there is no study in which MSR expression has been evaluated as a prognostic marker for prostate cancer before initial treatment. In the present study, we analysed immunohistochemically the expression of MSR in prostatic biopsy specimens and evaluated MSR as a prognostic marker.

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TABLE 1 The characteristics of the 135 patients

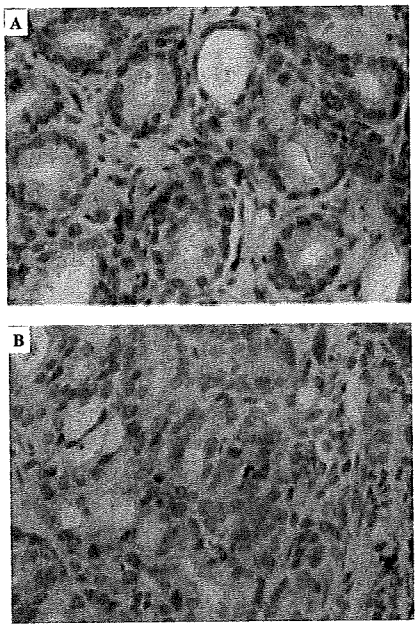
Characteristic (*n*)	Median (range) or *n* (%)
Age, years (135)	70 (50–88)
PSA level, ng/mL (135)	19.9 (4.3–5820)
Gleason score	
≤6	62 (45.9)
= 7	36 (26.7)
≥8	37 (27.4)
Clinical stage	
T1	24 (17.8)
T2	55 (40.7)
T3	53 (39.3)
T4	3 (2.2)

TABLE 2 Association between the MSR count and clinicopathological factors

Clinicopathological factor	*n* (%) of patients	Mean (SEM) MSR count	*P*
Age, years			
≤70	61 (45.2)	24.21 (13.08)	0.235
≥70	74 (54.8)	25.87 (13.40)	
PSA, ng/mL			
≤20	67 (49.6)	26.55 (12.83)	0.150
≥20	68 (50.4)	23.68 (13.56)	
Gleason score			
≤6	62 (45.9)	30.20 (12.93)	0.001*
= 7	36 (26.7)	22.43 (10.30)	<0.001†
≥8	37 (27.4)	19.17 (13.32)	0.124†
Clinical stage			
≤T2	79 (58.5)	27.42 (12.73)	0.027
≥T3	56 (41.5)	22.85 (13.30)	
PSA failure			
+	45 (33.3)	27.74 (13.96)	<0.001
–	90 (66.7)	19.84 (9.82)	

*≤6 vs =7; †≤6 vs ≥8; ‡ = 7 vs ≥8.

FIG. 1. MSR immunostaining of prostate cancer with a monoclonal antibody. A, high MSR staining; B, low MSR staining (×400).



PATIENTS AND METHODS

The present study included 135 patients who were diagnosed with prostate cancer at our hospital between 1997 and 2000. A written informed-consent form approved by our institute was obtained for this immunohistochemical study. The median (range) age of the men at admission was 70 (50–88) years. Prostate cancer was diagnosed by histopathological examination of specimens obtained by transrectal needle biopsy of the prostate. The clinical stage was based on the American Staging System (modified Whitmore-Jewett staging system) [17] using a DRE, TRUS, X-ray, CT, MRI and bone scintigraphy. The age, serum PSA level (measured by immunoenzymatic assay) and clinical stages of the 135 patients are shown in Table 1. Among the men, 70 were treated by radical prostatectomy (50) or radiotherapy (20) as a definitive therapy. There was no significant difference in the distribution of stage or Gleason score between those treated by radical prostatectomy or radiotherapy. The other 65 patients were treated with hormonal therapy because of high stage or advanced age. Biopsied specimens were fixed in 10% neutral-buffered formalin and routinely embedded in the paraffin; 5 μm sections were cut and stained with haematoxylin, and reviewed by a pathologist to determine the Gleason score, based on the Gleason grading system [18]. Patients were followed up with a periodic evaluation by a DRE, serum PSA level, and imaging findings. Recurrence of prostate cancer was defined as an increase in the serum PSA level on three consecutive measurements (PSA failure) or the appearance of new soft-tissue or metastatic lesions. A written informed-consent form approved by our institution was obtained from all patients for the histopathological study.

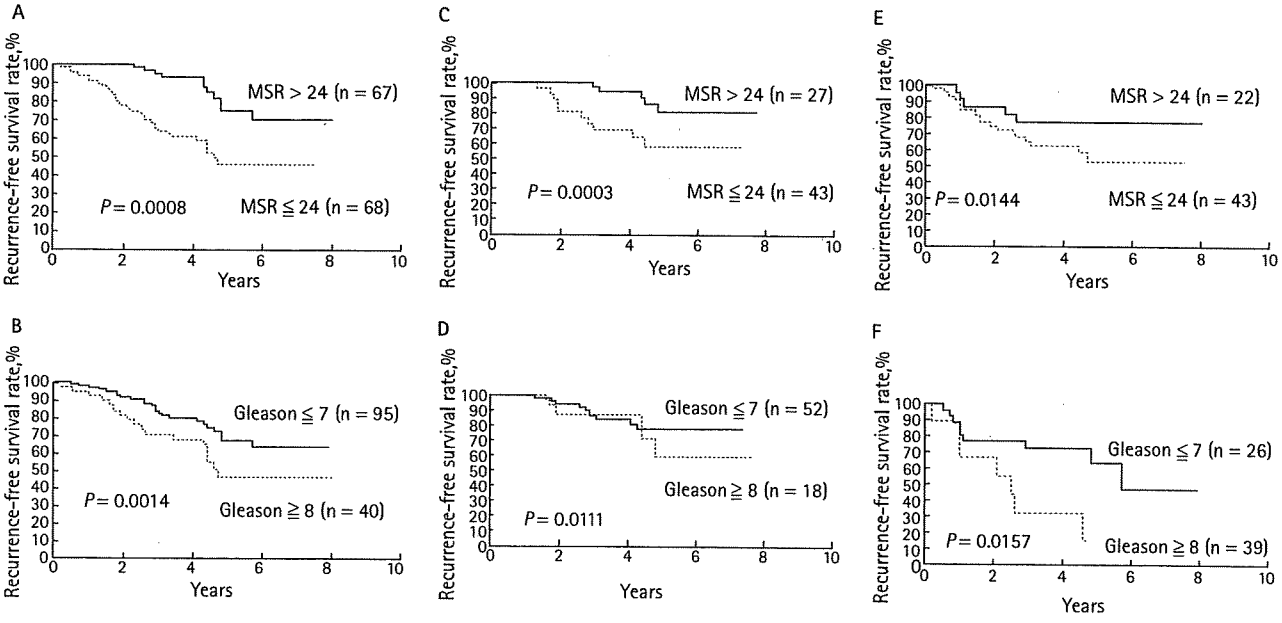
MSR was immunohistochemically labelled with a monoclonal antibody (CD204; Trans Genic, Inc., Kobe, Japan). Immunohistochemistry was carried out on paraffin sections by the LSAB method (Dako, Glostrup, Denmark). For quantification, six microscopic fields, each with an area of 0.06175 mm^2, were selected randomly at ×400 within each sample.

The correlation between MSR immunostaining and clinicopathological factors was evaluated using the chi-squared test and Fisher's exact probability test. Recurrence-free survival (RFS) rates were calculated using the Kaplan-Meier method, and differences in survival were analysed by the log-rank test. Independent prognostic factors were identified using the Cox proportional hazards regression model in a stepwise manner; in all tests, $P < 0.05$ was taken to indicate a statistically significant difference.

RESULTS

MSR-positive cells were detected in all specimens tested; most MSR-positive cells showed the morphological features of macrophages (Fig. 1). The median (range) number of MSR-positive cells (MSR count) was 24.0 (3.0–76.33). Of the 135 patients, 67 had an MSR count of >24 and were categorized as having a high level of MSR expression; the remaining 68 had an MSR count of <24 and were categorized as having a low level of MSR expression (Fig. 1A,B).

FIG. 2. RFS of patients with prostate cancer with: a high or low MSR count (A) and with a high or low Gleason score (B); significant differences between groups (A, P < 0.001; B, P = 0.001): With prostatic cancer treated by definitive therapy; C, with a high or low MSR count; and D, high or low Gleason score; significant differences between groups (C, P < 0.001; D, P = 0.011): With prostate cancer treated by hormonal therapy, E in patients with a high or low MSR count; and F with high or low Gleason score; significant differences between groups (E, P = 0.011; F, P = 0.016).



Relationships between the MSR count and various clinicopathological factors are shown in Table 2. Patients with a high PSA level (≥20 ng/mL) had lower MSR counts than those with a low PSA level (<20 ng/mL), but this difference was not significant ($P = 0.150$). Patients with high T stage cancer had lower MSR ($P = 0.027$). The MSR count was correlated positively with the Gleason score. Patients with PSA failure had significantly lower MSR counts than those without ($P < 0.001$).

The mean (range) follow-up was 46.8 (2.0–96.0) months; the RFS rate was significantly higher in those with higher MSR counts than in those with lower MSR counts ($P < 0.001$) (Fig. 2A). Patients with a high Gleason score (≥8) had a significantly lower RFS rate than those with a low Gleason score (<8; $P = 0.001$; Fig. 2B).

In patients treated by definitive therapy the RFS rate was significantly higher for those with high MSR counts than for those with low MSR counts ($P < 0.001$; Fig. 2C). Patients with a high Gleason score (≥8) had a significantly lower RFS rate than those with a low Gleason score (<8) ($P = 0.011$; Fig. 2D).

Androgen-deprivation therapy (ADT) is commonly used for prostate cancer in Japan, and therefore we analysed the RFS rate of 65 patients treated only with this therapy. The RFS rate was lower in those with low MSR counts and high Gleason scores than in those with high MSR counts and low Gleason scores ($P = 0.014$ and 0.016, respectively; Fig. 2E,F).

Results of multivariate analysis for all patients are shown in Table 3. Cox multivariate analysis showed that the MSR count is a significant prognostic factor ($P = 0.002$), in addition to Gleason score ($P = 0.011$), extraprostatic extension ($P = 0.038$), and distant metastasis ($P = 0.003$). When Cox multivariate analysis was applied to patients treated by definitive therapy or by hormonal therapy, MSR was also a significant prognostic factor ($P = 0.009$ and 0.007; Table 3).

DISCUSSION

MSR mRNA expression is up-regulated by macrophage colony-stimulating factor, and is down-regulated by interferon-γ, TNFα, TGF-β and interleukin-10 [19–21]. It was reported that inflammation and proliferative regeneration of the prostatic epithelium in the presence of increased oxidative stress is associated with MSR-1 expression in macrophages, and might be involved in the development of prostate cancer [9,22]. In the present study, low expression of MSR correlated significantly with a high Gleason score and high tumour stage. These findings are consistent with reports showing a correlation of high MSR-positive cells with an increased recurrence rate and poor prognosis of prostate cancer by analysis of surgical specimens [16]. In the present series, the RFS rate was significantly lower for patients with a lower MSR count and a higher Gleason score than for those with higher MSR and a lower Gleason score when the RFS was assessed for all patients. Similarly, the RFS rate was assessed in patients treated by definitive therapy (radical prostatectomy or radiotherapy); as expected, those with a higher MSR count had a better RFS rate than those with a lower MSR count. Multivariate analyses also showed that a lower MSR count in biopsy specimens was a significant prognostic factor, as was the Gleason score. From these data, we can predict PSA failure after definitive and hormonal therapy by the MSR count in the prostatic biopsy specimens. Although most patients are currently

TABLE 3 Prognostic factors in the Cox multivariate analysis for all patients, those receiving definitive therapy and those treated with hormonal therapy

Prognostic factor	Hazard ratio (95% CI), *P*		
	All patients	Definitive therapy	Hormonal therapy
MSR (>24)	2.959 (1.484–5.899), 0.007	0.958 (0.912–1.007), 0.009	2.594 (1.476–4.620), 0.007
PSA, ng/mL*	1.000 (0.998–1.001), 0.500	1.004 (0.992–1.016), 0.543	0.980 (0.949–1.011), 0.201
Gleason score (>7)	0.961 (0.932–0.991), 0.011	0.482 (0.126–1.85), 0.029	2.726 (1.969–5.034), 0.001
Extraprostatic extension (+)	1.347 (0.690–2.632), 0.038	2.377 (0.789–7.167), 0.012	0.998 (0.997–1.000), 0.065
Lymph node metastasis (+)	1.152 (0.225–5.888), 0.865	–	0.848 (0.170–4.231), 0.840
Distant metastasis (+)	14.30 (2.537–80.55), 0.003	–	43.29 (12.40–151.15), <0.001
DRE (+)	1.026 (0.483–2.175), 0.948	–	0.936 (0.455–1.927), 0.858

**Continuous variable.*

diagnosed with clinically localized disease, 30–40% fail to respond to local definitive therapy within 10 years, as evidenced by an increase in the PSA level [3,4]. ADT is widely used in Japan, particularly in older patients; the present series included 65 treated only with ADT. The RFS rate was better in patients with a higher MSR count than in those with a lower MSR count.

There have been several studies in which multivariate analyses showed the prognostic significance of stage, Gleason score and serum PSA level for prostate cancer [5,6,18]. In the present study, the MSR count was also a significant prognostic indicator for prostate cancer. The present multivariate analysis also showed the MSR count to be an independent prognostic indicator for RFS. Use of the MSR count in biopsy specimens allows a prediction of the prognosis of prostate cancer at the time of diagnosis.

By activating macrophages, granulocyte macrophage colony-stimulating factor enhances antitumour effector mechanisms [19]. The immune response is induced by immunisation with tumour cells genetically engineered to locally secrete cytokines [20]. Recently, various factors that lead to the progression-associated down-regulation of MSR expression have been reported [21,23]. MSR might be regulated by specific cytokines. TGF-β1 can inhibit MSR expression in the human monocyte/macrophage cell line THP-1 [23]. In humans, an increased TGF-β1 level is associated with prostate cancer progression and metastasis [24,25]. Interleukin-6 has been reported to inhibit MSR expression at the transcriptional level in THP-1 cells [26]. There is also evidence that the serum interleukin-6 level in patients with prostate cancer increases progressively with increased malignant potential, and is positively associated with metastasis [27,28]. It was reported that peripheral monocytes express MSR at a low level [29]. However, we showed that MSR expression in the prostate is a prognostic marker for prostate cancer. MSR might act locally as an antitumour effector.

In conclusion, MSR expression as determined by immunohistochemistry is a prognostic indicator for prostate cancer and might be used as an indicator for PSA failure after definitive therapy and for the response to hormonal therapy.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **MSR**, macrophage scavenger receptor; **RFS**, recurrence-free survival; **ADT**, androgen-deprivation therapy.

A retrospective analysis of ovarian endometriosis during pregnancy

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Objective: To clarify the frequency of pregnancy complicated by ovarian endometriosis and to investigate the size change and outcome of ovarian endometriosis during pregnancy.
Design: Retrospective study.
Setting: Departments of obstetrics and gynecology of the Osaka University and Izumiotsu Municipal Hospitals of Osaka, Japan.
Patient(s): Women who delivered between 1996 and 2007.
Intervention(s): None.
Main Outcome Measure(s): The frequency of pregnancies complicated by ovarian endometriosis and the size change of the lesions during pregnancy.
Result(s): The frequency of ovarian endometriosis-complicated pregnancy has almost quadrupled over the last 12 years, to become the most common adnexal mass detected during pregnancy; it was 0.14% (five cases among 3558 deliveries) during the 6-year period from 1996 to 2001, but has increased to 0.52% (19 cases among 3599 deliveries) during the 6-year period from 2002 to 2007, a statistically significant increase of 3.8-fold. Among 25 ovarian endometriotic lesions observed during pregnancy in 24 women (one case had two lesions), the size of the cyst decreased in 13 lesions (52%), went unchanged in 7 (28%), and increased in 5 (20%), that demonstrated decidualization, abscess and rupture.
Conclusion(s): Ovarian endometriosis during pregnancy can be safely observed conservatively; however, further investigation is required to predict the occurrence of abscess formation or rupture of ovarian endometriosis, and to distinguish the enlargements due to malignant transformation from those related to decidualization. (Fertil Steril® 2009;■:■–■. ©2009 by American Society for Reproductive Medicine.)

Key Words: Abscess, adnexal mass, decidualization, ovarian endometriosis, pregnancy, rupture

Endometriosis is an estrogen-dependent disease defined as the presence outside of the uterine endometrium of endometrial-like glandular and stromal tissue. Endometriosis, often associated with pelvic pain and infertility, is estimated to occur in 10% of the reproductive-age women in the United States, and the number of cases has been steadily increasing (1). Various genetic, embryogenic, immunologic, and environmental factors have been postulated in the etiology of endometriosis (2).

Assisted reproductive technology (ART) has recently made good progress against various causes of infertility, including endometriosis. The in vitro fertilization and embryo transfer (IVF-ET) technique is a useful therapeutic option to improve the pregnancy rate for women who are otherwise infertile as a consequence of severe endometriosis (3). As a consequence of the successes of ART, the number of pregnancies with an associated endometriosis is rising in parallel.

Because endogenous estrogen stimulates endometriosis, antihormonal therapy has been used to suppress the estrogen growth effect (2). Pseudo-pregnancy therapy using progestins, with or without estrogens, is one of the safest, most effective, and cost-beneficial treatments for non-pregnancy-related endometriosis. The long-standing clinical impression of the beneficial effect of a natural pregnancy on endometriosis has been voiced repeatedly. As Beecham stated, "Nature (since the beginning of time) has employed an efficient prophylactic and curative measure for endometriosis, i.e. pregnancy" (4). The observed suppressive effect of pregnancy on endometriosis was reviewed by McArthur and Ulfelder in 1965 (5). They showed that pregnancy was frequently accompanied by a reduction in the size of nonovarian endometriotic lesions, although there were notable exceptions. Because ultrasonography and other diagnostic procedures, such as computed tomography and magnetic resonance imagining, were not yet available, they could analyze only endometriotic lesions diagnosable by manual pelvic examination and inspection.

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Today, the so-called endometrioma of ovarian endometriosis is easily and routinely diagnosed by ultrasonography. The ovary is variously reported to be involved in 17% to 44% of endometriosis patients (6). Larger ovarian endometrioses seem to respond more poorly to medical therapy; however, very few studies have shown the effect of a purely medicinal therapy using drugs such as danazol or gonadotropin-releasing hormone agonist (GnRH-a) for endometriosis (7). The effects of an actual pregnancy on ovarian endometriosis in a large cohort of cases is also still largely unclear, although some cases of intra-abdominal bleeding caused by peritoneal endometriotic lesions during pregnancy have been reported (8–10).

A maternal adnexal mass, mainly an ovarian tumor, is one of the most severe complications that can occur during pregnancy; antepartum surgery is usually required to remove the mass. Approximately 1% to 4% of pregnant women are diagnosed with an ovarian mass, including a corpus luteum functional cyst. Of these rare pregnancy-associated masses, roughly 1% to 8% represent an even rarer malignant tumor. Ovarian endometriosis was reported to occupy only 5% to 6% of the adnexal masses detected during pregnancy. The most commonly encountered ovarian mass is a benign mature cystic teratoma (11–13).

We retrospectively analyzed the frequency of pregnancy complicated with ovarian endometriosis, diagnosed during the two consecutive periods 1996 to 2001 and 2002 to 2007. We also investigated during each pregnancy the size-change of the mass to clarify the effect of pregnancy on the ovarian endometriosis, and to verify whether the course of ovarian endometriosis can be observed safely during actual pregnancy.

MATERIALS AND METHODS

Patients

During the study period of 1996 to 2007, 7157 deliveries were performed within the departments of obstetrics and gynecology of Osaka University and Izumiotsu Municipal Hospitals of Osaka, Japan. The complications of the patients' adnexal masses were retrospectively reviewed using their clinical records, including physical examination notes, ultrasonography reports, operation records, and pathology reports. The general characteristics of pregnant women in the two hospitals did not different significantly, and the results were pooled.

Detection, Diagnosis, and Treatment of Adnexal Masses

As the standard of obstetric care in Japan, pelvic examination and ultrasonography are routinely performed by obstetrician/gynecologists in the first trimester for all pregnant women. Authorized specialists in obstetrics and gynecology, who had been trained in our institutes and who had shared similar practices, conducted transvaginal (and transabdominal) ultrasonography in the first trimester to measure the crown-rump length of fetuses and also to check for adnexal masses. Ultrasonography was performed throughout this study with a Mochida model Sonovista-ET (Mochida Co., Tokyo, Japan) using a 5-MHz transducer. A functional simple cyst that vanished before midpregnancy, a so-called corpus lutein cyst, was excluded from this study. Ultrasonographic criteria for diagnosis of ovarian endometriosis were [1] a cystic structure with a low, homogeneous echogenicity that [2] had a thick cystic wall with regular margins, as described in previous reports (14, 15). The maximum diameter of the cyst was measured in the ovarian endometriosis cases, and a change in the maximum diameter of the mass equal to or larger than 1 cm was regarded as significant in the current study.

Adnexal masses, except for corpus lutein cysts, ovarian endometriosis, and relatively small asymptomatic cystadenoma, were routinely surgically removed in the second trimester, except for four cases in which the patients rejected surgery during the pregnancy. Twenty-one out of 24 cases of ovarian endometriosis, in which surgical removal of the lesions was not performed, were diagnosed by their ultrasonographic features. In the remaining three cases, the diagnosis of ovarian endometriosis was made by pathology sections of the lesions removed surgically in the second trimester and at the time of cesarean section.

To tabulate the changes in frequency, composition, and outcomes of adnexal masses (including ovarian endometriosis) detected during pregnancy, the analysis of the number of deliveries and adnexal masses was split into the two consecutive periods: 1996 to 2001 and 2002 to 2007.

Analysis of the Size Change of Ovarian Endometriosis

Cases of suspected ovarian endometriosis detected in the first trimester were observered carefully by transvaginal ultrasonography (TV-US) every 4 to 8 weeks into the postpartum period. In the cases in which TV-US was insufficient to evaluate the cysts, transabdominal ultrasonography (TA-US) was also conducted. Both TV-US and TA-US was performed by authorized specialists of obstetrics and gynecology for subsequent follow-up evaluations. In 11 cases, the ovarian endometriosis was diagnosed before the onset of gestation. In eight cases, magnetic resonance imaging was performed to confirm the diagnosis of an adnexal mass during pregnancy or after delivery. This study was approved by the institutional review board and the ethics committee, and there were no conflicts of interest.

RESULTS

Complication of an Adnexal Mass during Pregnancy

During the two consecutive periods of 1996 to 2001 and 2002 to 2007, our hospitals combined to perform 3558 and 3599 deliveries, respectively (Table 1). The median age, the gravid/para status, and the rate of treatment of infertility in the former and the latter periods were 31 and 32, 2/1 and 2/1, and 4.2% and 5.0%, respectively, for the two periods, indicating that the characteristics of the pregnant women in the two periods were not statistically significantly different.

TABLE 1

Number of adnexal masses found during pregnancies.

	Earlier period (1996–2001)	Recent period (2002–2007)	Total period (1996–2007)
Delivery	3558	3599	7157
Adnexal mass	32 (0.90%)	49 (1.4%)	81 (1.1%)

Note: In the two different 6-year periods, from 1996 to 2001, and from 2002 to 2007, before delivery, 32 (0.90%) and 49 (1.4%) adnexal masses were detected during the pregnancy, respectively.

Ueda. Ovarian endometriosis during pregnancy. Fertil Steril 2009.

An adnexal mass was detected in 32 cases (0.90%) during the 1996 to 2001 period and in 49 cases (1.4%) during 2002 to 2007. In total, adnexal masses (excluding corpus lutein cysts) were diagnosed in a combined 1.1% of all the deliveries occurring between 1996 and 2007. The most common mass was a mature cystic teratoma, which occupied 41% of all the masses in the first 6-year period (Table 2), while ovarian endometriosis represented only 16% of the masses. However, in the most recent 6-year period studied, ovarian endometriosis became the leading adnexal mass detected during pregnancy, representing 39% of all the masses. The frequency of ovarian endometriosis rose from 0.14% to 0.52%, a 3.8-fold increase compared with the previous period (P=.007, Fisher's exact test).

The proportion of the other types of the masses showed no statistically significant change. Malignancy or borderline malignancy was detected in only three cases (0.042%) in the total deliveries of 11 years. These three malignancies represented only 3.7% of the adnexal masses detected during all these pregnancies. Torsion of the mass (defined as a rotation of more than 45 degrees on its long axis) was detected in three cases (3.7%); these cases consisted of a single case of mature cystic teratoma, one mucinous cystadenoma, and one paraovarian cyst. Rupture of the cystic mass was detected in the second trimester in a single case of ovarian endometriosis (case 5 in Table 3). An abscess was formed in the second trimester in one case (case 4).

Clinical Features and Size Changes of Ovarian Endometriosis during Pregnancy

Twenty-five adnexal masses present during the first trimester of pregnancy (in 24 patients, including case 6 with bilateral lesions) were diagnosed as ovarian endometrioses (Table 3). In two of these cases (cases 4 and 5), ART was used to achieve the pregnancy. Therapy with GnRH-a was administered to treat the endometriosis in two other cases (cases 11 and 21). The median age of the patients was 30.5 years (range: 26 to 40 years). The lesions were removed surgically in the second trimester in two cases (cases 1 and 2) and at the time of caesarian section in one case (case 6). Drainage of the abscess was performed in the second trimester in case 4. The endometriotic cyst ruptured in case 5.

The size of the 25 endometriotic masses was evaluated in 24 cases of ovarian endometriosis in which clinical data, including the sizes of the masses, were available. A size change

TABLE 2

Number (%) of each type of adnexal mass.

Type of tumor	Earlier period (1996–2001)	Recent period (2002–2007)	Total period (1996–2007)
Mature cystic teratoma	13 (41%)	13 (27%)	26 (32%)
Endometriotic cyst	5 (16%)	19 (39%)[a,b]	24 (30%)
Serous cystadenoma	6 (19%)	7 (14%)	13 (16%)
Mucinous cystadenoma	4 (13%)	5 (10%)	9 (11%)
Paraovarian cyst	3 (9%)	2 (4%)	5 (6%)
Struma ovarii	0 (0%)	1 (2%)	1 (1%)
Serous cystic tumor of borderline malignancy	0 (0%)	1 (2%)	1 (1%)
Serous cystadenocarcinoma	1 (3%)	0 (0%)	1 (1%)
Mucinous cystadenocarcinoma	0 (0%)	1 (2%)	1 (1%)

Notes: The types of adnexal tumors detected during pregnancy are shown in the order of their frequency. Mature cystic teratomas were the most common tumor type in the former period and endometriotic cyst the most common in the latter 6-year period.

[a] The frequency of endometriotic cyst during pregnancy increased statistically significantly (P=.007, Fisher's exact test).

[b] The proportion of endometriotic cyst in adnexal masses statistically significantly increased from 16% to 39% (P=.028, Fisher's exact test).

Ueda. Ovarian endometriosis during pregnancy. Fertil Steril 2009.

TABLE 3

Summary of the changes occurring in ovarian endometriosis during pregnancy.

Patient number	Patient age (years)	Treatment during pregnancy	Mode of pregnancy	Size (mm)				
				Before pregnancy	First trimester	Second trimester	Third trimester/postpartum	Change
1	27	SO	spontaneous	unknown	66	92	–	increase (decidualization)
2	31	SO	spontaneous	unknown	48	61	–	increase (decidualization)
3	33	obs	spontaneous	unknown	50	72	27	increase (decidualization) → decrease
4	40	drainage	ART	52	52	65	20 (after drainage)	increase (abscess)
5	35	drainage	ART	50	51	61	unknown	increase (rupture)
6	40	cystectomy	spontaneous	unknown	43/42	46/46	47/45	no change/no change
7	28	obs	spontaneous	unknown	41	43	41	no change
8	29	obs	spontaneous	unknown	23	20	21	no change
9	28	obs	spontaneous	33	30	32	30	no change
10	30	obs	spontaneous	42	43	40	40	no change
11	31	obs	post-GnRH-a	45	47	50	50	no change
12	30	obs	spontaneous	unknown	75	40	31	decrease
13	26	obs	spontaneous	unknown	80	85	69	decrease
14	27	obs	spontaneous	unknown	72	55	61	decrease
15	33	obs	spontaneous	unknown	63	50	53	decrease
16	27	obs	spontaneous	unknown	37	36	20	decrease
17	31	obs	spontaneous	unknown	39	40	19	decrease
18	29	obs	spontaneous	35	39	42	20	decrease
19	30	obs	spontaneous	41	42	30	25	decrease
20	33	obs	spontaneous	40	41	34	28	decrease
21	40	obs	post-GnRH-a	68	68	61	45	decrease
22	27	obs	spontaneous	40	40	39	10	decrease
23	33	obs	spontaneous	unknown	66	26	not detected	loss
24	35	obs	spontaneous	51	49	44	not detected	loss

Notes: For all 25 lesions of the 24 individual cases of ovarian endometriosis, the age of the patients, the treatment for their ovarian endometriosis, the mode of pregnancy inception, and the size change of the lesions during the pregnancy are shown. (/ = bilateral, left/right; – = removed ovary). ART: pregnancy by assisted reproductive technology; post-GnRH-a: pregnancy after treatment for endometriosis using gonadotropin-releasing hormone agonist; obs: observation; SO: salpingo-oophorectomy.

Ueda. Ovarian endometriosis during pregnancy. Fertil Steril 2009.

equal to or greater than 1 cm was regarded as a significant change.

In the first trimester, the ovarian lesion did not change in any of the 11 cases in which an ovarian endometriosis had already been diagnosed before the pregnancy (cases 4, 5, 9–11, 18–22, and 24). During the second trimester, the size of some of the 25 lesions began to change; the tumors increased significantly in size in five cases (cases 1–5) and decreased in five cases (cases 12, 14, 15, 19, and 23). The mass did not change in 14 cases (cases 6–11, 13, 16–18, and 20–23), which notably included the bilateral lesions of case 6.

In the third trimester and on into the postpartum period, the endometriotic lesions regressed in seven cases (cases 3, 13, 16–18, 21, and 22). In two cases the endometriotic cyst became dramatically undetectable (cases 23 and 24). Taken together, of the 25 ovarian endometriotic lesions (in 24 individual cases), the cyst size enlarged in only five lesions (20%), including a single lesion (case 3), which later regressed in the third trimester and postpartum period after undergoing a temporary enlargement in the second trimester. The tumor regressed in 13 cases (52%), including two lesions (4%) in which it vanished in the third trimester or the postpartum period; the tumor demonstrated size stability in seven lesions (28%). Decidualization (cases 1–3), abscess formation (case 4), and rupture during pregnancy (case 5) occurred in the five enlarging lesions. The initial size of the endometriotic cysts before pregnancy, or when first detected in the first trimester, was not related with the outcome of the lesions.

A preterm delivery was documented in only two cases, one for placenta previa and one for dichorionic diamniotic twins (cases 5 and 14). Other adverse effects of ovarian endometriosis on the pregnancy, including torsion of the cyst, were not detected.

Enlarging or Symptomatic Ovarian Endometriosis

Because a malignant transformation could not be ruled out, prepartum surgical removal of the enlarging adnexal mass was performed in two cases, both of which turned out to be benign decidualization (cases 1 and 2). In case 3, the cyst enlarged, and a carcinoma arising in the cyst could not be initially ruled out; however, the lesion demonstrated a probable decidualized pattern on ultrasonographic analysis. The patient rejected our recommendation of surgery, so the lesion was observed conservatively through her pregnancy. The tumor regressed during the third trimester and postpartum periods.

Peritoneal washing and drainage was performed in the two cases (cases 4 and 5) in which the lesions ruptured or became infected. The affected ovary could not be removed because of a severe adhesion of the cyst to the uterus and intestines in these cases.

CONCLUSION

In our current study, we have found that the frequency of ovarian endometriosis detected during pregnancy statistically significantly increased 3.8-fold during the 6-year period from 2002 to 2007 when compared with the 6-year period from 1996 to 2001 (P=.007, Fisher's exact test). One of the reasons for this dramatic increase of ovarian endometriosis during pregnancy has been previously proposed to be the result of the increasing overall frequency of endometriosis in patients (1). Our present study found that in the earliest 6-year period, from 1996 to 2001, the five pregnancies complicated with ovarian endometriosis were all unassisted (spontaneous) pregnancies (cases 1, 3, 6, 7, and 10), and that even in the more recent 6-year period, 2002 to 2007 when ART has been more widely used, the vast majority, 17 of 19 endometriosis-complicated pregnancies, were spontaneous ones (cases 2, 8, 9, and 11–24). These results may suggest that the remarkable success of using ART without removal of ovarian endometriosis to overcome previously untreatable infertility in severe cases of endometriosis (3) is not the major reason for the increase of pregnancy complicated with ovarian endometriosis. Better diagnosis of ovarian endometriosis may be another reason for the appearance of an increase of the disease during pregnancy; however, in the current study diagnosis was made using the same criteria throughout, and examination was performed by authorized specialists in obstetrics and gynecology using the same ultrasonography device throughout both study comparison periods.

The ovary is reported to be involved in roughly 17% to 44% of general endometriosis patients (6). Our present study shows that the size of these ovarian endometriotic cysts is increased during pregnancy in 20% of these cases, does not change in 28%, and decreases in 52%, providing the first relatively large-scale definitive description of the effects of pregnancy over time on ovarian endometriosis. These results also support the current effectiveness of the widely used pseudo-pregnancy therapies using progestins alone, or progestins and estrogens for ovarian endometriosis. However, large ovarian endometrioses have been reported to respond poorly to medical therapy (7). We found that the initial size of the cysts before pregnancy or in the first trimester was unrelated to size changes that occurred later during the pregnancy. The age of the patient also did not correlate with the size changes or outcomes of the ovarian endometriosis.

Several clinical cases of regressing tumors due to decidualization, infection, and rupture of ovarian endometriosis lesions during a pregnancy have been previously reported (16–23); however, the frequency of those incidences was far from clear. In our current study, among the 25 ovarian endometriotic cysts, decidualization, abscess, and rupture of the ovarian endometriosis were for the first time demonstrated to occur in 12%, 4%, and 4% of the cases, respectively. These effects were detected only in the enlarging cysts and were not detected in the stable or regressing cysts, except for a single case in which an abscess was formed 1 year after delivery (case 12). These results indicate that an enlarging ovarian endometriosis during pregnancy should be more carefully observed. However, three out of five enlarging ovarian

endometriosis lesions demonstrated potentially beneficial decidual changes in the second trimester. Among these, two lesions were removed surgically, but one lesion was observed conservatively throughout pregnancy, and the size of that lesion decreased in the third trimester and postpartum. Moreover, these endometriotic lesions of the ovary did not enlarge in the third trimester and postpartum. This result may imply that decidualized ovarian endometriosis may be observed conservatively during pregnancy. Although pseudo-pregnancy therapy is a widely performed treatment for endometriosis, this is the first detailed report of the changes in ovarian endometriosis observed during a relatively large number of actual pregnancies.

A previous study showed that intraobserver and interobserver standard deviations of ultrasonographic measurement of ovarian follicles from 1 to 3 cm in diameter were 0.06 cm and 0.12 mm, respectively (24); however, it is unclear how much difference in the diameter of pelvic cystic masses measured by ultrasonography can be regarded as significant. In our current study, where a change in the maximum diameter of the ovarian endometriotic cysts equal to or larger than 1 cm was regarded as significant, intraobserver or interobserver error could not be ruled out.

Ovarian endometriosis is histologically classified by the World Health Organization as a tumor-like lesion among ovarian tumors (25), which means it is a non-neoplastic lesion; however, recent molecular studies have shown that ovarian endometriosis is a monoclonal lesion (26, 27) that possesses genetic alterations similar to those of carcinomas, including the oncogenes *K-ras* and *Pten* mutations and loss of heterozygosity (LOH) at several sites (28–30). In our study, malignant transformation of the ovarian endometriosis was not detected during the pregnancy. However, clear cell and endometrioid adenocarcinomas have been reported to occur within ovarian endometriotic lesions (31). Malignant transformation is mostly detected in women over 40 years of age (32). However, a malignant transformation can occur during pregnancy (33–35), and the frequency of such events is on the rise simply because the mean age of pregnant women has increased due to delayed marriages caused by socioeconomic changes, especially in the more developed countries, including the United States and Japan (2). In fact, there were two pregnant women in our study who were 40 years old with complications due to ovarian endometriosis.

A malignant transformation of the tumor is highly suspected when a proliferative part is formed within the ovarian endometriotic cyst; however, a decidualized lesion can demonstrate very similar ultrasonographic features. In previous reports, the decidualized ovarian endometriosis demonstrated a relatively large round nonpapillary portion or a diffuse solid lesion in the cyst (17–19). On the other hand, adenocarcinoma arising within the ovarian endometriosis demonstrated a smaller papillary pattern (35). These ultrasonographic features may be used as possible indicators to discriminate malignant transformation from decidual change; however, a definitive noninvasive distinction between the two masses using ultrasonography is still impossible. A previous prospective study showed that a tumor size equal to or larger than 9 cm and postmenopausal status were independent predictive factors of patients for development of ovarian cancer from the ovarian endometriosis within several years (36). In our current study, there were no cases of ovarian endometriosis in which the cyst size was equal to or larger than 9 cm at first detection. In such cases of smaller tumors, our conclusion is that ovarian endometriosis may be observed conservatively during the pregnancy. These results also imply that ovarian endometriosis itself does not have to be surgically removed before pregnancy to avoid adverse effects during the pregnancy.

The number of pregnancies complicated with ovarian endometriosis will inevitably increase in the future, both because of the increase of endometriosis patients unrelated to pregnancy overall, and because of the progress being made in using ART to give these women viable pregnancies. Our study was a retrospective and limited one, and diagnosis of ovarian endometriosis was not made pathologically but rather was clinically based on ultrasonographic features in most cases; however, a high degree of diagnostic accuracy of ovarian endometriosis can be achieved by ultrasonography (7). Our findings provide supportive evidence, for the first time, for the widely accepted obstetric policy that surgical removal of an ovarian endometriosis is not necessary during pregnancy and will become an indicator for observation of ovarian endometriosis during these complicated pregnancies. Specific local or systemic prepartum hormonal changes including steadily rising levels of progestin during the pregnancy seem to cause a slowing or cessation of most cases of endometriosis. Further investigation is still required to clarify the varied mechanisms that underlie the third-trimester-induced decidualization of ovarian endometriosis. We will also seek ways to discriminate beneficial decidual changes from look-alike malignant transformation changes in enlarging ovarian endometriosis in hopes of finding ways to avoid unnecessary surgery during pregnancy and also to predict and prevent other adverse events, such as cyst rupture and abscess formation of ovarian endometriosis during pregnancy.

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Enhanced expression of Annexin A4 in clear cell carcinoma of the ovary and its association with chemoresistance to carboplatin

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Clear cell carcinoma (CCC) of the ovary is known to be highly resistant to platinum-based chemotherapy. The purpose of our study was to identify a candidate protein that is associated with chemoresistance of CCC and to investigate the specific mechanism of chemoresistance conferred by the identified protein. Enhanced expression of Annexin A4 (Anx A4) was identified in ovarian CCC cells using 2-D differential gel electrophoresis (2D-DIGE) and mass spectrometry. Anx A4 levels were elevated in CCC cells compared with non-CCC cells as determined by real-time RT-PCR and Western blot analysis. Immunohistochemical analysis of Anx A4 was performed in 126 epithelial ovarian cancer tissue samples and demonstrated significantly elevated levels of Anx A4 protein levels in ovarian CCC tumors compared with ovarian serous and endometrioid tumors ($p < 0.01$). Anx A4-transfected ovarian non-CCC cells were more resistant to carboplatin (IC50 = 42 μM) compared with control cells (IC50 = 23 μM) as determined by modified MTT assay. Intracellular platinum levels were significantly lower in Anx A4-transfected cells compared with control cells after carboplatin treatment ($p = 0.0020$) and after an additional 360 min of carboplatin-free incubation ($p = 0.0004$), as measured by atomic absorption spectrophotometry. Expression of Anx A4 is elevated in ovarian CCC tumors and is associated with chemoresistance in cultured ovarian cancer cells. These results demonstrate that Anx A4 confers chemoresistance in ovarian cancer cells in part by enhancing drug efflux. Thus, Anx A4 may represent a novel therapeutic target of chemoresistance in patients with ovarian CCC.

Key words: clear cell carcinoma of the ovary; chemoresistance; Annexin A4

Ovarian cancer is the 5th leading cause of cancer deaths for women in the United States, with approximately 21,600 new cases and 15,500 deaths reported annually.[1] In Japan, it is the eighth most common cause of cancer deaths, with approximately 7,700 new cases (2001) and 4,500 deaths (2007) reported yearly, and the incidence is increasing (Health, Labour and Welfare Ministry, Japan: Population Survey Report). More than 20% of all cases with ovarian cancer in Japan are classified as clear cell carcinoma (CCC) of the ovary, and for unknown reasons, this percentage is markedly higher (by approximately 2-fold) than in Europe and the United States.[2]

Because ovarian cancers (including ovarian CCC) are relatively asymptomatic at early stage, the majority of patients (approximately 70%) present with an advanced stage disease at first diagnosis and subsequently require surgical tumor reduction and adjuvant chemotherapy.[3,4] However, of the 4 major histological types of epithelial ovarian cancer, CCC of the ovary is highly resistant to conventional cancer chemotherapy (including carboplatin and paclitaxel treatment) compared with the other histological types.[2,5,6] Consequently, patients with ovarian CCC are associated with both poorer prognosis and higher mortality than patients with other types of ovarian cancer.[2] Thus, there is an urgent need to further our understanding of the pathogenesis of ovarian CCC, particularly with respect to the expression of proteins, which confer chemoresistance, for the development of a novel therapeutic strategy.

In this study, we performed a proteomic analysis using ovarian cancer cell lines [CCC for comparison with non-CCC serous adenocarcinoma (SAC)] to identify a candidate protein associated with chemoresistance in ovarian CCC. SAC was chosen as a control non-CCC cell line because of its chemosensitivity compared with the chemoresistant CCC cell line. We identified several proteins that are differentially upregulated in ovarian CCC compared with SAC and focused our investigation on Annexin A4 (Anx A4).

Anx A4 is an epithelial isoform of a ubiquitous family of soluble cytoplasmic proteins, which bind to and polymerize on the surface of cell membranes in response to increases in intracellular calcium.[7–9] Although the functions of Anx A4 have not been completely characterized, previous studies have identified major involvement of this protein in membrane permeability,[10] exocytosis[11,12] and regulation of ion channels.[13] Its roles in membrane fluidity and membrane trafficking may in part explain the involvement of Anx A4 in modulating drug resistance in cancer cells.

A previous report associating Anx A4 with chemoresistance in human cancer cell lines focused on human lung and colon cancer cell lines[14] but did not examine ovarian cancer cell lines. In addition, the mechanism of chemoresistance induced by Anx A4 has not been explored in detail. In the study of Morita *et al.*,[15] proteomic analysis showed enhanced expression of Anx A4 in the OVISE and OVTOKO ovarian CCC cell lines compared with the MCAS ovarian mucinous cancer cell line. However, a possible association between Anx A4 expression and chemoresistance was not investigated. Importantly, neither study tried to determine whether Anx A4 protein levels are elevated in tumors of patients with ovarian CCC.

In this study, we have addressed 2 important questions concerning Anx A4 and chemoresistance, *i.e.*, whether expression of Anx A4 is elevated in patient ovarian CCC tumors and by what mechanism Anx A4 confers chemoresistance.

Material and methods

Patients

We examined surgically obtained tumor tissue samples of 126 ovarian cancer patients (Table I) who underwent surgery at Osaka University Hospital, Japan, between 1999 and 2006. None of the patients entered in this study had received adjuvant chemotherapy, including paclitaxel or carboplatin treatment. Histologic features of the tissues were reviewed by board-certified pathologists. Diagnosis was based on the FIGO (International Federation of Gynecologists &

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