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 ($P = 0.06$).

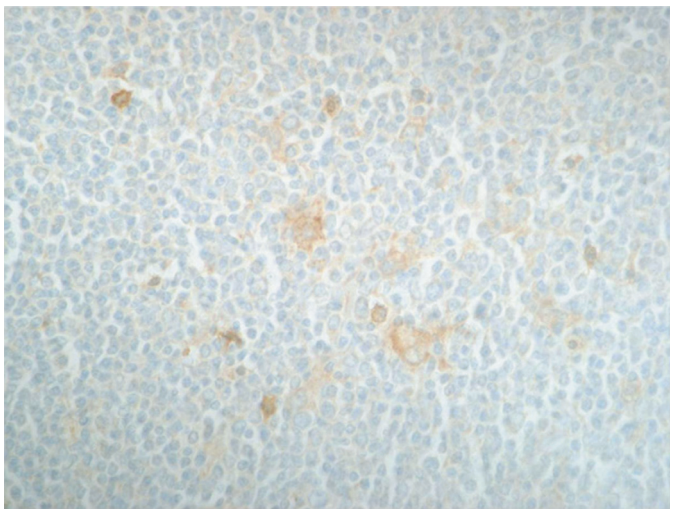

Fig. 1. Immunohistochemical analysis for vascular endothelial growth factor receptor (VEGFR) 1 in a pelvic lymph node. Clusters of VEGFR1-positive cells were identified in pelvic lymph nodes from patients with prostate cancer who underwent radical prostatectomy.

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.

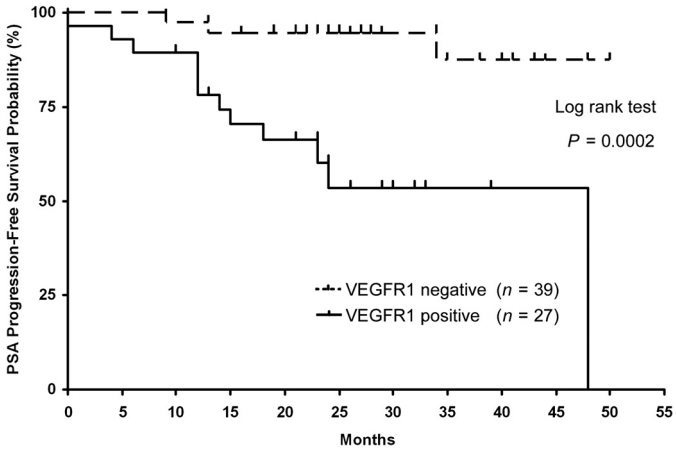

Fig. 2. Kaplan–Meier recurrence curves of biochemical progression-free probability for vascular endothelial growth factor receptor (VEGFR) 1-positive cases and VEGFR1-negative cases in 66 patients without lymph node metastasis and with negative surgical margin. PSA, prostate-specific antigen.

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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