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Significance of phospho-vascular endothelial growth factor receptor-2 expression in pancreatic cancer

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Vascular endothelial growth factor receptors (VEGFRs) are mainly expressed by endothelial cells, but they are also expressed by some cancer cells, including pancreatic cancer. The objective of this study was to evaluate the significance of VEGFRs expression in pancreatic cancer cells. A total of 107 primary pancreatic tumors were stained with antibodies against VEGFR-1, VEGFR-2, phospho-VEGFR-2 (pVEGFR-2), VEGFR-3, VEGF-A, VEGF-C, and VEGF-D. VEGFR-2 and pVEGFR-2 expression were positive in 74 (69%) and 54 (50%) of 107 pancreatic cancers. There was a significant correlation ($P < 0.001$) between VEGFR-2 expression and pVEGFR-2 expression. pVEGFR-2 was significantly associated with invasion to the anterior capsule of pancreas ($P = 0.032$) and arterial invasion ($P = 0.012$). In contrast, VEGFR-1 and VEGFR-3 expression was only observed in 13 (12%) and 15 (14%) of 107 pancreatic cancers, and was not associated with any clinicopathological features. The prognosis of pVEGFR-2 positive patients with stage IIA tumors was significantly ($P = 0.0441$) poorer than that of pVEGFR-2-negative patients. VEGF-A, VEGF-C, and VEGF-D expression was positive in 42 (39%), 82 (77%), and 39 (36%) of 107 pancreatic cancers, respectively. The prognosis for VEGF-A-positive patients was significantly ($P = 0.0425$) poor, but not for VEGF-C-positive and VEGF-D-positive patients. A multivariate analysis indicated pVEGFR-2 expression to be an independent prognostic factor, but not VEGF-A. These findings suggested that VEGFR-2 signaling might therefore be associated with the prognosis of patients with pancreatic cancer. The expression of pVEGFR-2 might be a novel predictive prognostic marker for patients with pancreatic cancers, especially at clinical stage IIA. (*Cancer Sci* 2010; 101: 1529–1535)

Pancreatic cancer is one of the most lethal solid tumors of the gastrointestinal tract. Although the management and treatment of patients with pancreatic cancer has improved in the past few decades, the overall 5-year survival rate remains at less than 5%.[1] Long-term survival is rare even in patients who undergo a histologically curative operation, with the overall 5-year survival rates ranging from 10% to 25%.[2,3] The high mortality rate of pancreatic cancer is due to extensive invasion into surrounding tissues and metastasis to distant organs at the time of diagnosis or even after a curative operation; however, the molecular mechanisms remain unclear.[4]

A number of studies have shown an increased expression of vascular endothelial growth factor (VEGF), and a potent mitogen for endothelial cells at the primary site, to be correlated with a poor prognosis for various tumors including pancreatic cancer.[5] Recent studies have demonstrated that VEGF-A expression at the primary site is correlated with metastatic ability in pancreatic cancer.[6] These results indicate that VEGF-A expression is an important predictor for both distant metastasis and poor prognosis in ductal pancreatic adenocarcinoma. VEGFs specifically interact with receptor tyrosine kinases, VEGFR-1, -2, -3. These receptors are mainly expressed by endothelial cells, but they are also expressed by some cancer cells.[4,5,7–11] Many studies have previously concluded that angiogenesis by VEGF receptors (VEGFR) was responsible for the poor prognosis of various tumors.[5,11] On the other hand, VEGFs demonstrate not only mitogens for endothelial cells but also the presence of invasion-stimulating activity for some types of cancer cells, such as ovarian,[4] bladder,[10] and colorectal cancers.[11] Therefore, VEGF might not only stimulate tumor angiogenesis of endothelial cells but also be capable of directly affecting pancreatic cancer cell motility through VEGFR. Although VEGFR expression in cancer cells seems to be an important risk factor for patients with pancreatic cancer, only a few studies have shown a relationship between the expression of VEGFR and prognosis in pancreatic cancer.[12–14]

This study examined the correlation between clinicopathological features and VEGFR-1, -2, -3 expression in human pancreatic cancer. This study provided clinical evidence that the VEGFR-2 expression in cancer cells correlates significantly with invasion into the surrounding tissues as well as with poor prognosis of pancreatic cancer.

Materials and Methods

Clinical materials. A total of 107 patients who had undergone resection of a primary pancreatic tumor at our institute, and who were histologically confirmed to have pancreatic cancer, were enrolled in the present study. The pathologic diagnoses and classifications were made according to the International Union Against Cancer (UICC) Classification of Malignant Tumors.[15] Histological findings were according to the classification of pancreatic carcinoma by the Japan Pancreas Society.[16] The median follow-up time for all 107 patients was 18.3 months (range, 3–129 months). The patients' tumor characteristics are shown in Table 1. The survival curve shows Kaplan–Meier overall survival curves in relation to VEGFR-1, VEGFR-2, VEGFR-3, phospho-VEGFR-2 (pVEGFR-2), VEGF-A, VEGF-C, and VEGF-D expression levels in pancreatic cancers. The survival curve was calculated from the date of surgery.

Antibodies and reagents. Mouse monoclonal antibodies which recognize VEGFR-1 (clone RR9S, sc74007), VEGFR-2 (clone A-3, sc6251), VEGFR-3 (clone MM0003-7G63, sc101562), VEGF-A (clone A-20, sc152), VEGF-C (clone F-10, sc74585), and VEGF-D (clone MM0007-7E79, sc101584) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit monoclonal antibody which recognizes phospho-VEGFR-2 (Tyr951) was purchased from Cell Signaling, (Cell Signaling, Danvers, MA, USA). Normal mouse immunoglobulin G biotinylated rabbit antimouse immunoglobulin G, normal rabbit immunoglobulin G biotinylated yagi antirabbit immunoglobulin G, streptavidin–peroxidase reagent, and diaminobenzidine were purchased from Nichirei (Tokyo, Japan).

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Table 1. Patient clinicopathological characteristics

Variable	n = 107
Gender	
Male	63
Female	44
Age (years)	66.15
T category	
T1	4
T2	15
T3	80
T4	8
N category	
N0	39
N1	66
M category	
M0	93
M1	14
Stage	
I	9
II	75
III	16
IV	4
Histological type	
Differentiated	62
Undifferentiated	36

Immunohistochemical techniques. The methods for the immunohistochemical determination of VEGFR-1, VEGFR-2, pVEGFR-2, VEGFR-3, VEGF-A, VEGF-C, and VEGF-D are described in detail in the manufacturers' instructions. Briefly, tumor specimens were fixed in 10% formaldehyde solution and embedded in paraffin. Four-micrometer-thick sections were cut and mounted on glass slides. The slides were deparaffinized in xylene. The tissues were heated for 20 min at 105°C and at 0.4 kg/cm^2 by autoclave in Target Retrieval Solution (Dako, Carpinteria, CA, USA). The sections were then dewaxed and incubated with 3% hydrogen peroxide in methanol for 15 min. Next, the sections were incubated in 10% normal rabbit serum for 10 min. The specimens were incubated with the VEGFRs or VEGFs antibodies (1:1000) overnight at 4°C. Sections were incubated with biotinylated rabbit antimouse immunoglobulin G for 30 min. Slides were treated with streptavidin–peroxidase reagent and were incubated in 3, 3′- diaminobenzidine for 1 min, counterstained with Mayer's hematoxylin.

Immunohistochemical determination of VEGFR and VEGF staining. The tumor specimens showed various staining patterns against anti-VEGFR and anti-VEGF antibody. VEGF and VEGFR expression was analyzed according to the percentage of cells showing membrane positivity, that is, staining as strong as that seen in the normal epithelium: 0, 0–10%; 1+, 10–20%; 2+, 20–50%, 3+, >50%. The degree of monoclonal antibody reactivity in individual tissue sections was considered positive if unequivocal staining of the membrane was seen in more than score 2+. The slides were interpreted by two investigators without knowledge of the correspondence to clinicopathological data.

Statistical analysis. We used the chi squared, Fisher's exact, or Mann–Whitney U-tests to determine the significance of the differences between the covariates. Pearson correlation coefficient analysis was calculated to determine relations. Survival durations were calculated using the Kaplan–Meier method and were analyzed by the log-rank test to compare the cumulative survival durations in the patient groups. The Cox proportional hazards model was used to compute univariate and multivariate hazards ratios for the study parameters. For all tests, a P-value of <0.05 was defined as statistically significant. The SPSS software program (SPSS Japan, Tokyo, Japan) was used for the analyses.

Results

Correlation between clinicopathological features and VEGFR expression. VEGFR-1, VEGFR-2, VEGFR-3, and pVEGFR-2 were mainly expressed at the cell membrane and partly the cytoplasm of pancreatic cancer cells, especially at the invading tumor edge (Fig. 1). VEGFR-2 and pVEGFR-2 expression was positive in 74 (69%) and 54 (50%) of 107 pancreatic cancers, while VEGFR-1 and VEGFR-3 expression was observed in only 13 (12%) of 107 and 15 (14%) of 107, respectively. There was a significant positive correlation between VEGFR-2 and pVEGFR-2 expression (r = 0.553, P < 0.001). The relationship between the VEGFR expression and the clinicopathological features of the pancreatic tumors are shown in Table 2. VEGFR-2 expression was significantly associated with invasion to the anterior capsule of the pancreas (P = 0.017) and retroperitoneum (P = 0.027); and pVEGFR-2 expression was significantly associated with invasion to the anterior capsule of the pancreas (P = 0.032) and arterial invasion (P = 0.012). In contrast, no association was observed between VEGFR-1 or VEGFR-3 expression and other clinicopathological features.

Correlation between clinicopathological features and VEGF expression. VEGF-A were expressed at the cytoplasm of pancreatic cancer cells (Fig. 1). VEGF-A, VEGF-C, and VEGF-D expression was positive in 42 (39%), 82 (77%), and 39 (36%) of 107 pancreatic cancers, respectively. The relationship between VEGF expression and clinicopathological features of the pancreatic tumors are shown in Table S1. Significant correlation was found only between VEGF-D expression and lymphnode metastasis (P = 0.040); however, no correlation was observed between VEGF-A or VEGF-C expression and other clinicopathological features.

Survival. The prognosis for VEGFR-2- and pVEGFR-2-positive patients was significantly poorer than that for VEGFR-2- and pVEGFR-2-negative patients (P = 0.0098 and P = 0.0432, Fig. 2). The 5-year survival of patients with VEGFR-2-positive tumors was 0% in comparison to 21% for patients with negative tumors. The VEGFR-1 and VEGFR-3 levels did not correlate significantly with patient survival (Fig. 2). The prognosis of pVEGFR-2-positive patients with stage IIA tumors was significantly (P = 0.0441) poorer than that of pVEGFR-2-negative patients, while no significant difference in the prognosis was

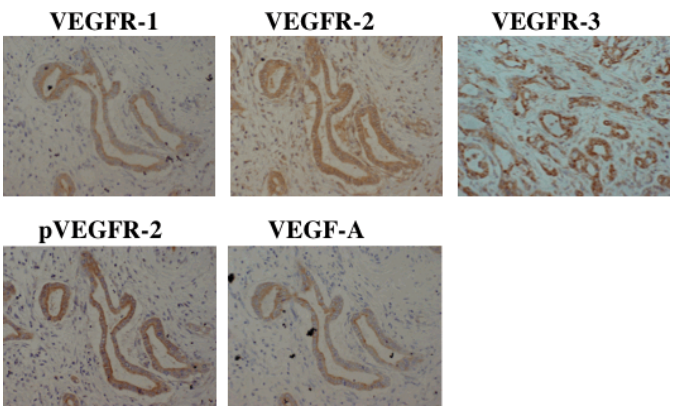

Fig. 1. Vascular endothelial growth factor receptor (VEGFR) and VEGF-A expression at the invading tumor front of pancreatic cancer. VEGFR-1, -2 and pVEGFR-2 and -3 were mainly expressed at the cell membrane and partly in the cytoplasm of pancreatic cancer cells (×200). VEGF-A was expressed in the cytoplasm of pancreatic cancer cells.

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Table 2. Correlation between VEGFR expression and clinicopathological features in 107 patients with pancreatic cancer

Characteristics	VEGFR-1			VEGFR-2			pVEGFR-2			VEGFR-3		
	Positive	Negative	*P*-value	Positive	Negative	*P*-value	Positive	Negative	*P*-value	Positive	Negative	*P*-value
Total	13 (12%)	94		74 (69%)	33		54 (50%)	53		15 (14%)	92	
Age (years)	66 ± 9	66 ± 11		66 ± 11	67 ± 11		66 ± 10	66 ± 11		70 ± 6	66 ± 11	
Gender												
Male	8 (13%)	55	0.835	40 (63%)	23	0.129	31 (49%)	32	0.755	5 (8%)	58	0.3
Female	5 (11%)	39		34 (77%)	10		23 (52%)	21		10 (23%)	34	
T category												
T1, T2	3 (16%)	16	0.698	15 (79%)	4	0.308	13 (68%)	6	0.084	5 (26%)	14	0.89
T3, T4	10 (11%)	78		59 (67%)	29		41 (47%)	47		10 (11%)	78	
N category												
Negative	6 (15%)	33	0.473	27 (69%)	12	0.83	21 (54%)	18	0.703	7 (18%)	32	0.41
Positive	7 (10%)	59		47 (20%)	19		33 (50%)	33		8 (12%)	58	
M category												
M0	11 (12%)	82	0.678	64 (69%)	29	0.844	49 (53%)	44	0.236	13 (14%)	80	0.975
M1	2 (14%)	12		10 (71%)	4		5 (36%)	9		2 (14%)	12	
Stage												
I	1 (11%)	8	0.856	6 (67%)	3	0.253	6 (67%)	3	0.382	3 (33%)	6	0.66
II	9 (12%)	67		54 (71%)	22		40 (53%)	36		8 (11%)	68	
III + IV	3 (15%)	17		14 (70%)	6		8 (40%)	12		4 (20%)	16	
Histological type												
Differentiated	5 (14%)	31	0.728	26 (72%)	10	0.156	17 (14%)	19	0.822	5 (14%)	31	0.492
Undifferentiated	5 (8%)	57		43 (69%)	19		33 (8%)	29		8 (13%)	54	
Anterior capsular invasion												
Negative	5 (11%)	42	0.649	38 (81%)	9	0.017	29 (62%)	18	0.032	6 (13%)	41	0.905
Positive	8 (14%)	51		35 (59%)	24		24 (41%)	35		8 (14%)	51	
Retroperitoneal invasion												
Negative	2 (5%)	36	0.229	32 (84%)	6	0.027	24 (63%)	14	0.066	6 (16%)	32	0.522
Positive	11 (16%)	56		40 (60%)	27		11 (16%)	56		8 (12%)	59	
Liver metastasis												
Negative	9 (10%)	82	0.605	58 (64%)	33	0.161	43 (47%)	48	0.605	9 (10%)	82	0.43
Positive	0 (0%)	5		5 (100%)	0		3 (60%)	2		1 (20%)	4	
Peritoneal dissemination												
Negative	12 (11%)	93	0.229	73 (70%)	32	0.524	54 (11%)	51	0.229	13 (12%)	92	0.019
Positive	1 (50%)	1		1 (50%)	1		0 (0%)	2		2 (100%)	0	
Lymphatic invasion												
Negative	3 (17%)	15	0.405	13 (72%)	5	0.79	9 (50%)	9	1.000	3 (17%)	15	0.412
Positive	8 (10%)	76		58 (69%)	26		42 (50%)	42		10 (12%)	74	
Arterial invasion												
Negative	9 (11%)	71	0.471	56 (70%)	24	0.105	44 (55%)	36	0.012	11 (14%)	69	0.766
Positive	1 (6%)	17		9 (50%)	9		4 (22%)	14		7 (39%)	11	
Venous invasion												
Negative	8 (13%)	52	0.529	41 (68%)	19	0.877	29 (48%)	31	0.777	9 (15%)	51	0.425
Positive	4 (9%)	39		30 (70%)	13		22 (51%)	21		5 (12%)	38	

TNM classification is according to the International Union against Cancer (UICC, 2002). pVEGFR-2, phospho-vascular endothelial growth factor receptor-2; VEGFR-1, vascular endothelial growth factor receptor 1.

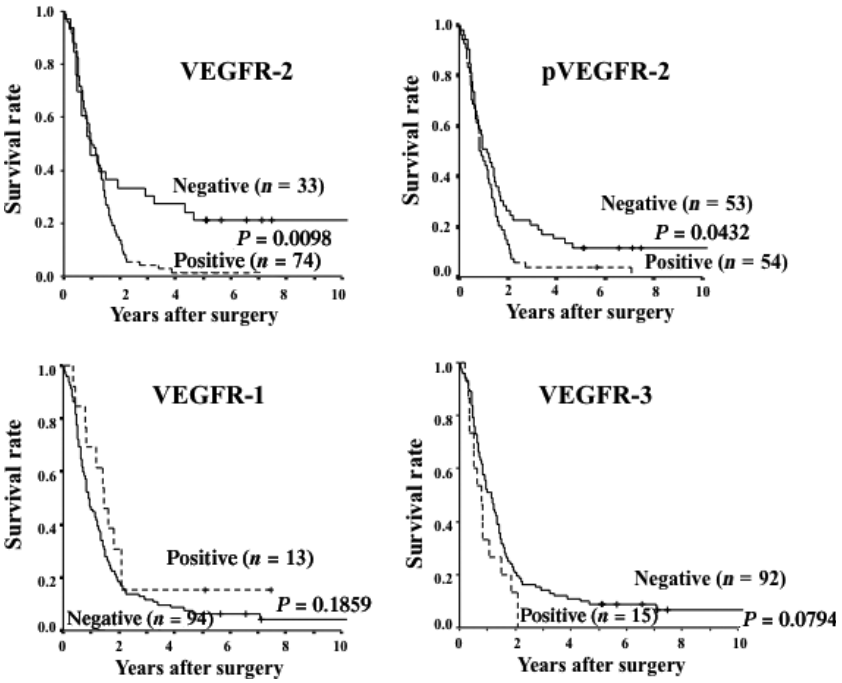

Fig. 2. The overall survival of patients based on vascular endothelial growth factor receptor (VEGFR)-based analysis. The survival curve shows Kaplan–Meier overall survival curves in relation to the VEGFR levels in the pancreatic cancer. The prognosis of all 107 patients with VEGFR-2-positive ($P = 0.0098$) or pVEGFR-2-positive tumors ($P = 0.0432$) was significantly worse than that of those with VEGFR-negative tumors. In contrast, there was no association between VEGFR-1 or VEGFR-3 expression and overall survival.

found between VEGFR-2 expression in stages I, IIB, or III + IV (Fig. 3a). The prognosis for pVEGFR-2-positive patients was significantly poorer among 88 patients who underwent a curative R0 resection, than that of VEGFR-2-negative patients ($P = 0.0168$); and the prognosis of pVEGFR-2-positive patients who underwent curative R0 resection with stage IIA tumors was significantly ($P = 0.0428$) poorer than that of pVEGFR-2-negative patients, while no significant difference in prognosis was found among pVEGFR-2 expression in stage I (Fig. 3b). The prognosis for VEGF-A-positive patients was significantly poorer than that for VEGF-A-negative patients ($P = 0.0425$), while VEGF-C and VEGF-D were not significantly associated with the patient survival (Fig. 4). A univariate analysis revealed the presence of VEGFR-2 expression, pVEGFR-2 expression, VEGF-A expression, liver metastasis, peritoneal dissemination, and portal vein invasion to all be significantly correlated with patient survival (Table 3). A multivariate analysis showed pVEGFR-2 expression, peritoneal dissemination, and portal vein invasion to all be significantly independent prognostic factors, but not VEGF-A (Table 4).

Discussion

This study investigated the expression of the VEGFR receptors in pancreatic cancer cells in parallel with histopathological parameters and prognosis. There are only a few reports that VEGF receptors are expressed by pancreatic cancer cells.[12–14] VEGFR-2 was markedly overexpressed in pancreatic cancer cells, but only weakly in the normal pancreatic duct cells. The present study showed that pVEGFR-2 expression was high in 50% of pancreatic cancers, but VEGFR-1 and 3 was low in around 10%. This shows that VEGFR-2 is not a vasculature-restricted receptor, but has an additional role in cancer cell biology itself in about half pancreatic cancers. Pancreatic cancer with the presence of pVEGFR-2-positive cancer cells was histologically associated with extra-pancreatic invasion, thus suggesting that VEGFR-2 activation plays a role in the higher invasion levels of pancreatic cancer cells. In contrast, a relationship with clinicopathologic parameters was not seen for VEGFR-1 and VEGFR-3 expression, suggesting that VEGFR-1 and VEGFR-3 signaling might not be associated with invasion ability.

In our preliminary study using five pancreatic carcinoma cell lines, we found that VEGFR-2 was expressed in pancreatic cancer cell lines, and VEGF-A significantly increased the motility of pancreas cancer cells, which was inhibited by *VEGFR-2* siRNA. Moreover, the VEGFR-2 phosphorylation level of pancreas cancer cells was increased by VEGF-A, and decreased by VEGFR-2 inhibitors (data not shown). These *in vitro* data and the current immunohistochemical results suggest that VEGF-A/VEGFR-2 signaling might play an important role in the invasion of pancreatic cancer cells.

A multivariate analysis showed VEGFR-2 to be an independent factor of prognosis in pancreatic cancer. The prognosis of stage IIA patients with VEGFR-2-positive tumors was significantly worse than that of those with VEGFR-2-negative tumors, while no significant difference in prognosis at stages I, IIB, III, and IV was found in VEGFR-2 expression. These findings suggested that the expression of VEGFR-2 might therefore be a useful predictive factor in pancreatic cancer, especially at clinical stage IIA. Lymph node metastasis has already developed at stages IIB, III, and IV. VEGFR-2 signaling might affect the prognosis of patients without distant metastasis. The numbers of patients with clinical stage I and III + IV disease might be insufficient for the estimation of statistical difference in this study, because patients with pancreatic cancer at stage I are rare and most patients with stage III + IV are inoperable. Although no significant correlation between prognosis and VEGFR-2 expression was recognized in patients with clinical stage I or III + IV disease, large numbers of patients with clinical stage I and III + IV disease might be necessary to conclude the significance of VEGFR-2 in patients with stage I or III + IV disease.

VEGF-A, -C, -D bind VEGFR-2.[17] There is a relationship between VEGF-A, -C, -D and the prognosis of patients with pancreatic cancer because of angiogenesis and lymphangiogenesis due to VEGFR signaling expressed in endothelial cells.[5] Although VEGF-A activity has been mostly focused on the vascular endothelium, it is conceivable that VEGF increases tumor

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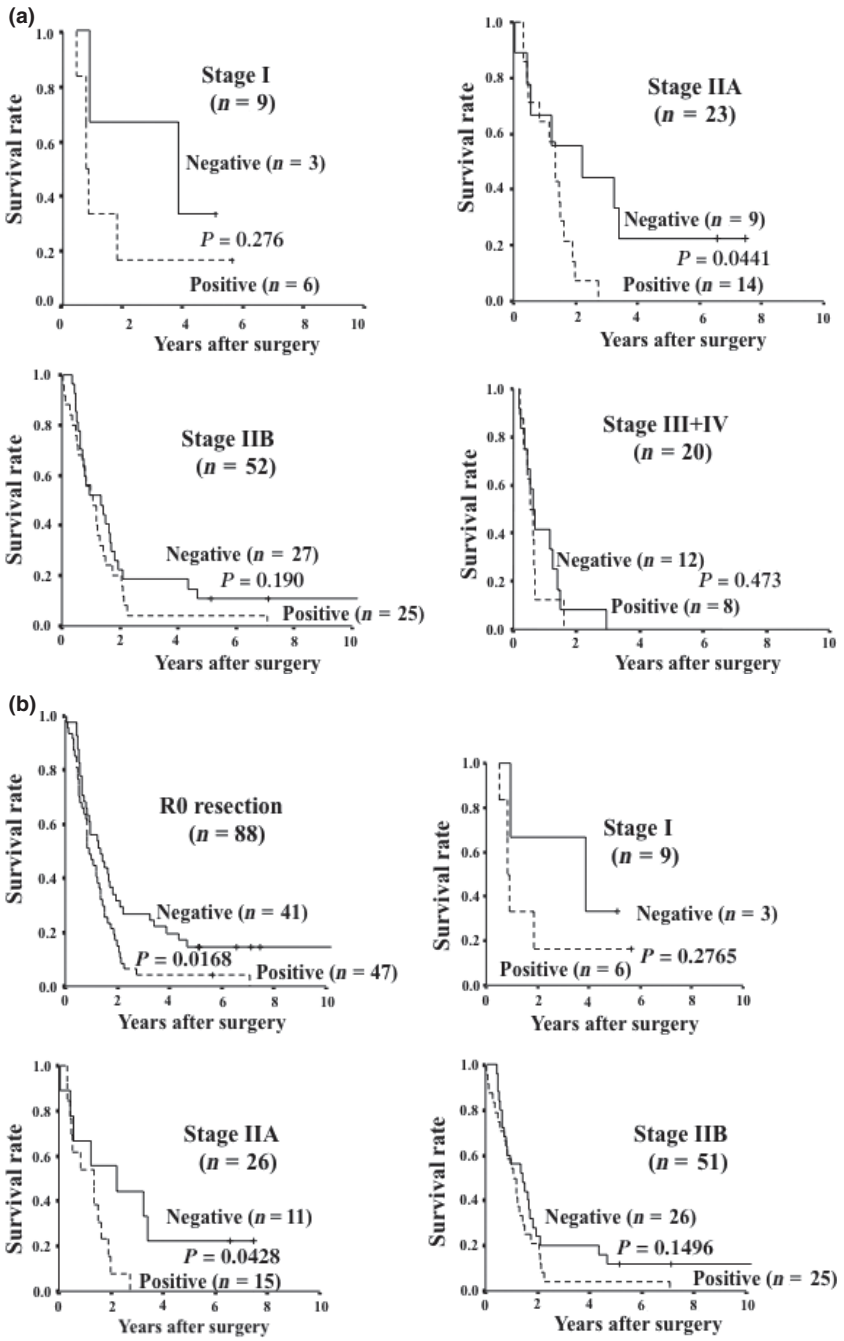

Fig. 3. The overall survivals stratified for phosphovascular endothelial growth factor receptor-2 (pVEGFR-2) expression in cancer cells according to the status of curative resection or clinical stage. (a) Overall survivals in 107 patients with pancreatic cancer according to clinical stage. Overall survivals of the subgroups of 107 patients were subdivided according to the status of clinical stage. The prognosis of pVEGFR-2-positive cancer was significantly poorer ($P = 0.0441$) than that of p-VEGFR2-negative cancer in the stage IIA groups. (b) The overall survivals in the 88 patients with a curative R0 resection, the prognosis of the pVEGFR-2-positive patients ($n = 47$) was significantly ($P < 0.05$) worse than that of the 41 patients who were pVEGFR-2-negative. The prognosis of pVEGFR-2-positive cancer with a curative R0 resection was significantly poorer ($P = 0.0428$) than that of p-VEGFR2-negative cancer in the stage IIA groups.

progression not only by stimulating tumor angiogenesis but also by direct stimulation of VEGFR signaling in various types of tumor cells.[18–22] In this study, there is a relationship between VEGF-A and the prognosis of patients with pancreatic cancer, but not VEGF-C and VEGF-D. The recent discovery of pVEGFR-2 in pancreas tumor cells and the close correlation between VEGF-A expression and poor prognosis might suggest the significance of an autocrine VEGFA/VEGFR-2 pathway in pancreatic cancer cells.

Various types of therapy including chemotherapy, hyperthermia, and immunotherapy have been tested for effectiveness in pancreatic carcinoma, but none has been satisfactory. The development of a molecular targeting drug might be important as a treatment against invasion of pancreatic cancer. Accordingly, novel therapies based on the characteristic biologic behavior of pancreas cancer are urgently sought. Our results suggest that VEGF/VEGFR-2 signaling is associated with cancer cell invasion and prognosis in pancreatic cancer. VEGF- or VEGFR-2-targeted therapy including receptor-specific antibodies and low molecular weight chemicals such as bevacizumab (Avastin),[23] or SU11248 [24] and KRN951 [25] may enhance the efficacy of standard therapy for pancreatic cancer.

Several studies have reported a number of growth factor receptors, including VEGFR-1,[13] c-Met,[26] transforming growth factor-beta1 receptor (TGF-β1R),[27,28] and fibroblast growth factor receptor 2 (FGFR-2),[29] to possibly contribute to the invasive aggressiveness of pancreatic cancer cells. This study demonstrated a correlation between the VEGFR-2 expression in

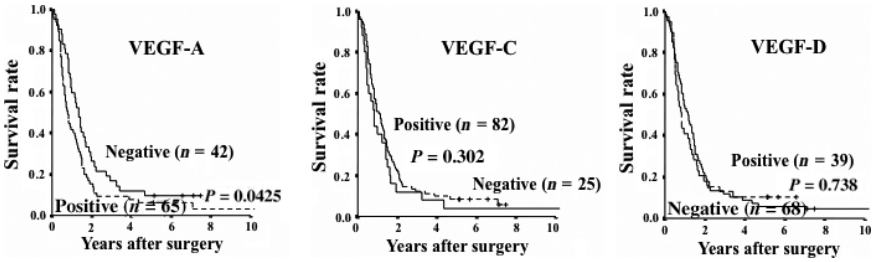

Fig. 4. The overall survival of patients based on vascular endothelial growth factor (VEGF)-based analysis. The survival curve shows Kaplan–Meier overall survival curves in relation to the VEGF levels in the pancreatic cancer. The prognosis of all 107 patients with VEGF-A tumors was significantly ($P = 0.0425$) worse than that of those with VEGF-negative tumors. In contrast, there was no association between VEGF-C or VEGF-D expression and overall survival.

Table 3. Univariate analysis with respect to overall survival in 107 patients with pancreatic cancer

	Risk ratio	95% Confidence interval	*P*-value
VEGFR-1			
Positive *vs* negative	0.647	0.345–1.213	0.190
VEGFR-2			
Positive *vs* negative	1.894	1.166–3.075	0.011
VEGFR-3			
Positive *vs* negative	1.667	0.957–2.907	0.083
pVEGFR-2			
Positive *vs* negative	1.507	1.009–2.250	0.045
VEGF-A			
Positive *vs* negative	1.517	1.011–2.276	0.044
VEGF-C			
Positive *vs* negative	0.786	0.496–1.244	0.304
VEGF-D			
Positive *vs* negative	1.074	0.711–1.624	0.734
Histological type			
Undifferentiated *vs* differentiated	1.178	0.886–1.568	0.26
Lymph node metastasis			
Positive *vs* negative	1.245	0.822–1.886	0.349
Liver metastasis			
Positive *vs* negative	3.888	1.536–9.844	0.004
Portal vein invasion			
Positive *vs* negative	1.771	1.168–2.684	0.004
Peritoneal dissemination			
Positive *vs* negative	10.97	2.414–49.843	0.001

pVEGFR-2, phospho-vascular endothelial growth factor receptor-2; VEGFR-1, vascular endothelial growth factor receptor 1.

Table 4. Multivariate analysis with respect to overall survival in 107 patients with pancreatic cancer

	Risk ratio	95% Confidence interval	*P*-value
pVEGFR-2			
Positive *vs* negative	1.569	1.002–2.458	0.049
VEGF-A			
Positive *vs* negative	1.372	0.885–2.128	0.169
Liver metastasis			
Positive *vs* negative	4.249	1.551–11.821	0.005
Portal vein invasion			
Positive *vs* negative	1.869	1.179–3.011	0.008
Peritoneal dissemination			
Positive *vs* negative	5.866	1.042–33.016	0.045

pVEGFR-2, phospho-vascular endothelial growth factor receptor-2; VEGF-A, vascular endothelial growth factor A.

pancreatic cancer cells and tumor invasion. These results might therefore be important in regard to the development of a molecular targeting drug to determine a key signal of invasion among these receptors.

In conclusion, the expression of VEGFR-2 in cancer cells was found to be significantly associated with the prognosis of patients with pancreatic cancer. The expression of VEGFR-2 might be a novel predictive prognostic marker for patients with pancreatic cancers, especially in clinical stage IIA patients.

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Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Correlation between vascular endothelial growth factor A (VEGF-A), VEGF-C, VEGF-D expression and clinicopathologic characteristics of patients. VEGF-D was only associated with lymph node metastasis. VEGF-A and VEGF-C were not associated with any clinicopathologic characteristics.

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