Serum Vascular Endothelial Growth Factor/Soluble Vascular Endothelial Growth Factor Receptor 1 Ratio Is an Independent Prognostic Marker in Pancreatic Cancer

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Objectives: Tumor angiogenesis is the consequence of an imbalance between positive and negative angiogenic regulatory factors. We sought to determine the role of pretreated serum angiogenic factors, including vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and soluble vascular endothelial growth factor receptor 1 (sVEGFR-1), in predicting clinical outcome in patients with pancreatic cancer.

Methods: We assessed pretreated serum VEGF, PlGF, and sVEGFR-1 levels in 92 patients with pancreatic adenocarcinoma and 60 healthy control subjects using an enzyme-linked immunosorbent assay. The correlation between these angiogenesis-related factors and clinicopathologic factors, including staging and overall survival, was analyzed.

Results: Serum levels of VEGF, PlGF, and sVEGFR-1 were significantly higher in patients with pancreatic cancer compared with those in controls (583.8 ± 559.5 vs 187.63 ± 393.32, 17.65 ± 7.34 vs 10.93 ± 1.21, and 50.94 ± 51.17 vs 15.55 ± 1.98 pg/mL, respectively; $P < 0.0001$). A reverse correlation was observed between sVEGFR-1 level and the advance of tumor stage. Cox regression analysis showed that the VEGF/sVEGFR-1 ratio was an independent predictor for pancreatic cancer survival. Higher VEGF/sVEGFR-1 ratio was significantly correlated with poor outcome in patents with pancreatic cancer.

Conclusions: Vascular endothelial growth factor/sVEGF-1 ratio is an independent prognostic factor for survival in pancreatic cancer. Its significance should be assessed when considering antiangiogenic therapy in treating pancreatic cancer patients.

Key Words: Vascular endothelial growth factor, placental growth factor, soluble vascular endothelial growth factor receptor 1, soluble fms-like tyrosine kinase 1, pancreatic adenocarcinoma, prognosis

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Pancreatic adenocarcinoma is the most common epithelial exocrine pancreatic neoplasm with a poor survival rate. Despite the advances in the research of the molecular pathogenesis, pancreatic adenocarcinoma remains a major unsolved health problem. Overall, the 5-year survival rate is less than 5%, and only approximately 20% of the patients with resectable disease survive 5 years.[1–3] Most of the patients with pancreatic cancers are diagnosed with advanced diseases.[4] One of the factors related to treatment failure is the high potential to develop metastasis and local recurrence. Chemotherapy is not regarded to have satisfactory results in treating pancreatic cancer, and novel approaches are required. Angiogenesis is crucial in the proliferation and metastasis of pancreatic cancers.[5] Inhibitors of angiogenesis are under extensive investigation, and several prospective trials have been devoted to treat pancreatic cancer.[6,7] To date, the role of the antiangiogenic therapy in pancreatic cancer is promising, but the results are not convincingly superior to the standard chemotherapeutic treatments.[8]

Angiogenesis is essential for tumor growth and development of metastasis. The angiogenic phenotype depends on a net balance between positive and negative angiogenic factors. Vascular endothelial growth factor (VEGF) is known to be a major regulator of angiogenesis in a variety of tumors, including pancreatic cancer.[9–12] The VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF).[13,14] Three VEGF receptors (VEGFRs) have been identified: VEGFR-1 (Flt-1), VEGFR-2 (KDR, Flk-1), and VEGFR-3 (Flt-4).[15] Vascular endothelial growth factor is both chemotactic and mitogenic for endothelial cells and acts to increase the permeability of the vascular endothelium. The correlation of VEGF expression and tumor progression or poor survival of pancreatic cancer has been reported,[11,12,16,17] some of them showing that the expression of VEGF is correlated with survival.[11,16,18] On the contrary, some of the reports in the literature did not support the prognostic role of VEGF in pancreatic cancer.[19–21] Placental growth factor was reported to be related to the prognosis in patients with colorectal cancer by our groups and with breast caner by Parr et al.[22,23] The role of PlGF in pancreatic cancer has not been studied before. Vascular endothelial growth factor receptor 1, expressed on endothelial cells and on macrophages, has been described as a positive and negative regulator of VEGF signaling capacity.[24] Soluble VEGFR-1 (sVEGFR-1, sFlt-1), an alterative splicing variant

TABLE 1. Clinical Demographic Data and Serum Level of VEGF, PlGF, and sVEGFR-1 in Pancreatic Cancer (PC) Patients and Controls

Parameters	Patients (n = 92)			Controls (n = 60)
Sex (M/F)	46/46			30/30
Age	65.00 ± 12.75			65.00 ± 7.47
	Total	Resectable PC	Advanced PC	
VEGF*	583.8 ± 559.5	498.07 ± 556.85	660.13 ± 565.67	187.63 ± 393.32
PlGF*	17.65 ± 7.34	17.13 ± 4.78	18.06 ± 8.87	10.93 ± 1.21
sVEGFR-1*†	50.94 ± 51.17	56.53 ± 20.55	46.71 ± 16.43	15.55 ± 1.98

Values of serum VEGF, PlGF, and sVEGFR-1 were presented as mean ± SD; age was presented as median ± SD.
*$P < 0.0001$ between pancreatic cancer patients and control group.
†$P < 0.05$ between respectable pancreatic cancer patients and advanced pancreatic cancer patients.
F indicates female; M, male; NS, not significant; PC, pancreatic cancer; $P < 0.05$, statistically significant.

of the VEGFR-1, is believed to be a modulator or a negative counterpart of the VEGF signaling pathway.[25,26] Previous studies have shown that sVEGFR-1 was present in the sera of healthy individuals,[27] and sVEGFR1 plays a significant role in the regulation of angiogenesis by binding competitively to VEGFs as a natural VEGF inhibitor.[28] It is thought that sVEGFR1 may function to reduce or modulate VEGF or PlGF activity in physiological and pathophysiological angiogenesis. Vascular endothelial growth factor receptor 1, including its soluble form sVEGFR1, is involved in a variety of human illnesses, making it an important target in the development of new strategies to treat disease. An inhibitory role of sVEGFR-1 in pancreatic cancer angiogenesis resulting in tumor suppression has recently been reported.[29] In addition, antiangiogenic gene therapy using sFlt-1 vector can effectively inhibit angiogenesis in hepatocellular carcinoma and ovarian cancer.[30,31] Although elevated serum levels of these factors have been observed in various types of human cancer,[32,33] little is known regarding their roles and clinical significance in patients with pancreatic cancer. In this study, we measured the serum levels of VEGF, PlGF, and sVEGFR-1 in patients with pancreatic cancer and in healthy controls, then we evaluated the correlations between these levels and clinicodemographic characteristics in patients with pancreatic cancer.

MATERIALS AND METHODS

Study Population

A total of 92 consecutive patients diagnosed with pancreatic cancer based on histologically or cytologically (by endoscopic ultrasonography–guided fine needle aspiration) proved ductal adenocarcinoma at the Department of Internal Medicine and Surgery, National Taiwan University Hospital, Taiwan, between January 1999 and December 2006 were enrolled. No patient had an active infection or inflammatory disease at the time of collection or had received blood transfusion, radiotherapy, or chemotherapy before the enrollment. Patients underwent Whipple operation in operable disease or received primary chemotherapy with gemcitabine in patients with advanced pancreatic cancer. Follow-up was performed (at least 3-month intervals) and included history, physical examination, hemogram, standard biochemical panel, and proper radiological examination. The clinicopathologic features of the patients, including TNM staging, were recorded. A fasting morning blood sample before treatment was obtained for VEGF, PlGF, and sVEGFR-1 assay. The control group consisted of 60 sex-matched healthy volunteers (median age, 64 years; 30 men and 30 women) in whom the absence of neoplastic disease was established by clinical history, physical examination, routine blood tests, urine and stool occult blood tests, chest x-ray examination, abdominal ultrasonography, and follow-up for more than 3 years. The study was approved by the ethics committee of the National Taiwan University, and informed consent was obtained from all patients and control subjects.

Blood Sample Collection and Measurement of Angiogenesis-Related Factors in Serum

Ten milliliters of peripheral venous blood was obtained at fasting early in the morning in a serum separator tube, and the sera were immediately separated by centrifugation at 3000 *g* for 10 minutes in a refrigerated centrifuge. The sera were stored at −80°C until analysis by a technician who was blinded to the patients' condition. The levels of sVEGFR-1, VEGF, and PlGF in the serum were assayed by a quantitative sandwich enzyme-linked immunosorbent assay method as previously reported (Quantikine; R&D Systems, Inc, Minneapolis, Minn) in duplicate according to the manufacturer's protocol.[34] The results were expressed as picograms per milliliter.

Statistical Analysis

The serum levels of each angiogenic factors were summarized as mean and standard deviation and then compared across subgroups of patients on the basis of stage, nodal status, and distant metastasis status using the Mann-Whitney-Wilcoxon test. Correlations were calculated with Spearman correlation test. Exploratory analysis was performed using proportional hazards model with binary indicators based on tentative cutoffs or its median value. Patients who were alive at last follow-up or died as a result of causes other than pancreatic cancer were censored at the date of last follow-up, and overall survival time was estimated using the Kaplan-Meier product limit method and analyzed by log-rank test. A univariate model was constructed

TABLE 2. The Clinical Characteristics of Patients With Pancreatic Cancer and Correlation With Serum Level of VEGF, PlGF, and sVEGFR-1

	n	VEGF, pg/mL	PlGF, pg/mL	sVEGFR-1, pg/mL	Survival, mo
TNM stage					
I	11	531.09 ± 756.18	19.35 ± 6.15	70.86 ± 28.73	14.67 ± 4.75
II	37	519.40 ± 530.19	17.32 ± 7.64	49.20 ± 15.67	7.55 ± 0.77
III	2	540.04 ± 21.73	9.00 ± 8.12	45.65 ± 0.78	5.00 ± 4.24
IV	42	670.94 ± 542.45	17.90 ± 7.28	47.25 ± 15.36*	3.87 ± 3.70*
Lymph node involvement					
Negative	43	581.27 ± 743.47	16.65 ± 5.53	58.29 ± 25.12	10.73 ± 6.83
Positive	49	613.59 ± 442.28	17.15 ± 7.98	49.09 ± 13.88†	4.00 ± 4.85†
Distant metastasis					
Negative	50	522.79 ± 568.56	17.44 ± 7.46	53.82 ± 20.81	8.17 ± 1.63
Positive	42	670.94 ± 542.45	17.90 ± 7.28	47.25 ± 15.36	3.87 ± 3.70‡

Value presented as mean ± SD of VEGF, PlGF, and sVEGFR-1; survival presented as median ± SD.
*$P < 0.05$ compared with stages I to III.
†$P < 0.05$ compared with negative lymph node involvement.
‡$P < 0.05$ compared with no metastasis.

to examine the hazard ratio of patient characteristics and prognostic factors individually. A multivariate Cox regression model was used to determine independent prognostic factors for death from pancreatic cancer. A $P < 0.05$ was considered statistically significant. Data analyses were performed using SPSS software (SPSS 11; Chicago, Ill).

RESULTS

Serum Angiogenic Factors in Pancreatic Cancer Patients Are Higher Than Those in Controls

The clinical characteristics of 92 patients with pancreatic cancer and 60 controls and the levels of serum VEGF, PlGF, and sVEGFR-1 in patients with pancreatic cancer and healthy controls were shown in Table 1. The median serum concentrations of VEGF, PlGF, and sVEGFR-1 in patients with pancreatic cancer were statistically significantly higher than the concentrations detected in the healthy group ($P < 0.001$; Table 1). There was no statistically significant correlation among the serum levels of VEGF, PlGF, and sVEGFR-1 in patients with pancreatic cancer (VEGF vs PlGF: $\rho = 0.05$, $P = 0.619$; VEGF vs sVEGFR-1: $\rho = 0.131$, $P = 0.236$; PlGF vs sVEGFR-1: $\rho = 0.146$, $P = 0.177$).

Angiogenic Factor (VEGF, PlGF, sVEGFR-1) Levels in Pancreatic Cancer Patients and Their Relation to Clinicopathologic Features

Serum levels of sVEGFR-1 correlated significantly with disease stage with lower sVEGFR-1 levels detected as the disease stage increased ($P < 0.0001$; Table 2). The patients with lymph node involvement also had significantly lower sVEGFR-1 levels in comparison with patients without lymph node involvement ($P = 0.000$; Table 2). However, there was no significant difference regarding VEGF and PlGF levels to the advance of disease ($P > 0.05$; Table 2).

Correlations Between VEGF, PlGF, sVEGFR-1, and VEGF/sVEGFR-1 Ratio and Patient Survival

Univariate analysis showed TNM stage, local T stage, distant metastasis, and preoperative VEGF/sVEGFR-1 ratio were significant factors affecting overall survival (Table 3). In the multivariate Cox proportional hazards model, in addition to stage, only preoperative VEGF/sVEGFR-1 ratio predicted survival independently (hazard ratio, 1.032; 95% confidence interval, 1.007–1.056; $P = 0.01$; Table 4). The median survival time was 7.8 months for the patients with preoperatively

TABLE 3. Univariate Analysis for Prognostic Factors of Survival in Patients With Pancreatic Cancer

Parameter	HR	95% CI	*P*
Age	1.005	0.98–1.02	0.629
Sex	0.945	0.67–1.47	0.801
TNM stage (I vs II vs III vs IV)	1.42	1.16–1.74	0.001*
T (T1 vs T2 vs T3 vs T4)	1.93	1.35–2.76	0.000*
Nodal status (N0 vs N1)	2.20	1.30–3.72	0.003*
Distant metastasis (M0 vs M1)	2.12	1.31–3.42	0.002*
Serum VEGF level	1.00	1.00–1.001	0.094
Serum PlGF	0.98	0.94–1.004	0.217
Serum sVEGFR-1	0.99	0.98–1.002	0.102
VEGF/sVEGFR-1 ratio	1.03	1.007–1.053	0.03*

*$P < 0.05$; statistically significant.
CI indicates confidence interval; HR, hazard ratio.

TABLE 4. Multivariate Analysis for Prognostic Factors of Survival in Patients With Pancreatic Cancer

	HR	95% CI	*P*
Age	1.002	0.98–1.009	0.873
Sex	1.196	0.71–2.007	0.497
TNM stage (I vs II vs III vs IV)	0.345	1.08–0.67	0.008*
sVEGFR-1	0.997	0.98–1.025	0.603
VEGF/sVEGFR-1 ratio	1.032	1.007–1.056	0.01*

*Statistically significant.
CI indicates confidence interval; HR, hazard ratio.

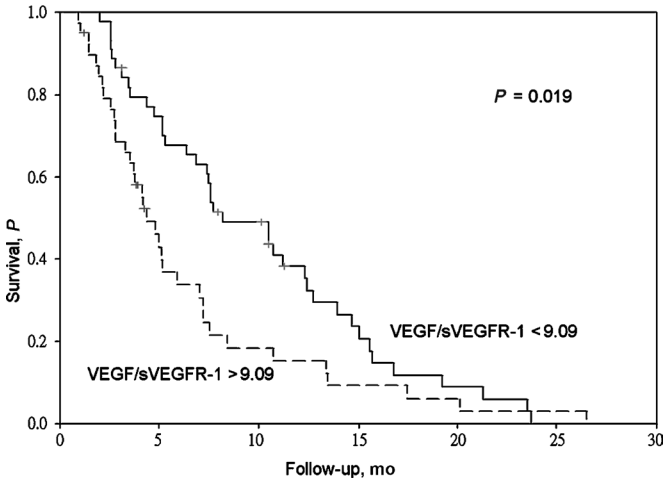

FIGURE 1. Kaplan-Meier survival curve of all patients with pancreatic adenocarcinoma grouped based on the VEGF/sVEGFR-1 ratio. The difference between groups was significant ($P = 0.019$, log-rank test).

VEGF/sVEGFR-1 ratio lower than 9.09 (the median of the entire sample of pancreatic cancer patients) and 4.10 months for those with a higher VEGF/sVEGFR-1 ratio (>9.09). There is a significant difference in overall survival between the 2 groups ($P = 0.019$; Fig. 1). There is no correlation between serum VEGF or PlGF concentrations and overall survival in patients with pancreatic cancer.

DISCUSSION

In this study, we have shown that serum levels of VEGF, PlGF, and sVEGFR-1 in pancreatic cancer were higher than those in healthy controls, and sVEGFR-1 correlated inversely with advanced stage of disease. In addition, the VEGF/sVEGFR-1 ratio is an independent prognostic factor of pancreatic cancer. To our knowledge, the current study is the first to show the clinical significance of serum VEGF/sVEGFR-1 ratio in pancreatic cancer.

In previous studies, the role of serum level of VEGF or tissue VEGF expression as a prognostic marker in pancreatic cancer is unsettled.[35] Our findings are in agreement with previous studies in the aspect of much higher serum VEGF concentration in patients with pancreatic cancer.[17,18] Although a trend of progressive elevation of serum VEGF with an advance in overall stage was found in this study, it did not reach statistical significance. Besides, no correlation of serum VEGF level to patient survival was shown in our study. One of the factors might be the wide distribution of the serum VEGF levels in our patients. In addition, many other proangiogenic factors are involved in the angiogenic process of pancreatic cancer. The prognosis of patients with pancreatic cancer is probably determined by the interaction or effects of multiple angiogenic factors instead of VEGF alone. Placental growth factor is implicated in several pathological processes, including the growth and spread of cancer. Our group has reported that PlGF expression is correlated with the survival of patients with colorectal cancer.[23] In this study, we found that serum levels of PlGF in patients with pancreatic cancer were higher than those in controls. However, there was no correlation between the serum levels of PlGF and the stage of disease or patient survival. The role of higher serum PlGF in pancreatic cancer needs further studies.

The function of VEGFR-1 remains unclear in the process of angiogenesis. Previous studies showed that VEGFR-1 had both positive and negative regulatory function in angiogenesis.[36–38] Soluble VEGFR-1, an alterative splicing variant of the VEGFR-1, is believed to be a modulator or a negative counterpart of the VEGF signaling pathway by binding to VEGF with high affinity and inhibiting VEGF's mitogenic response.[25,26] The sVEGFR-1 might be secreted from endothelial cells and monocytes, and it can be present in serum or plasma in healthy controls.[27] It is not very clear what role it plays during physiological or pathological angiogenesis. It was proposed that sVEGFR-1 might interact with the PlGF/VEGF heterodimers then competitively inhibit the activation of VEGFRs by VEGF homodimers.[39] Previous studies have shown that sVEGFR-1 is expressed in breast cancer, astrocytic tumors, and colorectal cancer and is correlated with tumor growth and prognosis.[40–42] In addition, studies of transfer sVEGFR-1 gene in the in vitro studies and animal models demonstrated an inhibition of tumor growth in pancreatic, hepatocellular, ovarian, and colon cancers.[29,30,43,44] In our study, we found that sVEGFR-1 was elevated in pancreatic cancer patients compared with normal controls. Furthermore, we found that the serum level of sVEGFR-1 was inversely correlated with the progression of pancreatic cancer. Our results, together with previous reports, suggest that decreased serum levels of sVEGFR-1 might significantly contribute to an aggressive phenotype in different cancers, including pancreatic cancer. Further studies to elucidate the role and mechanism of sVEGFR-1 in pancreatic carcinogenesis are needed.

There are a lot of studies to use biomarkers for assessing angiogenesis with correlation to cancer patients' outcome. The measurement of circulating factors is a convenient and noninvasive method that is potentially applicable to every cancer patient.[45] A recent study of the relationship between circulating sVEGFR-1 levels and preeclampsia showed that increased sVEGFR-1 is significantly associated with the development of preeclampsia, suggesting that immunodetectable sVEGFR-1 is biologically active.[46] Because angiogenesis is controlled between angiogenic and antiangiogenic factors, it is reasonable to use the ratio of VEGF/sVEGFR-1 to correlate with clinical outcome such as in preeclampsia.[47] Vascular endothelial growth factor/sVEGFR-1 ratio has been reported to be correlated to clinical outcome of cancer patients in myeloproliferative disorder, acute myeloid leukemia,[48,49] astrocytic tumors,[41] and breast cancer.[40,50] We found that the lower ratio of VEGF/sVEGFR-1 predicted a better prognosis in pancreatic cancer patients. These observations imply that the balance between VEGF and sVEGFR-1 might be crucial in cancer progression, and the ratio of serum level is a better predictor of patients' outcome instead of VEGF or sVEGFR-1 alone.

In summary, our study demonstrated that sVEGFR-1 is inversely correlated with the stage of the disease in patients

with pancreatic cancer. Furthermore, the ratio of VEGF/sVEGFR-1 is an independent biomarker for the pancreatic cancer patient's survival. Antiangiogenic therapy against VEGF and its receptors has been widely applied in the management of patients with cancer. In the future, more novel antiangiogenic agents and new combinations will be tested in clinical trials. As shown in our study, VEGF and sVEGFR-1 are involved in the angiogenesis of pancreatic cancer and are associated with treatment response and patient survival. Serum levels of VEGF and sVEGFR-1 should be measured and might be helpful to lead an efficient individualized antiangiogenic therapy for patients with pancreatic cancer in the future.

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