Association between Expression of Transcription Factor Sp1 and Increased Vascular Endothelial Growth Factor Expression, Advanced Stage, and Poor Survival in Patients with Resected Gastric Cancer

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ABSTRACT

The biological and clinical behaviors of cancer are affected by multiple molecular pathways that are under the control of transcription factors. Improved understanding of how transcription factors affect cancer biology may lead to improved ability to predict clinical outcome and discovery of novel therapeutic strategies. We evaluated the relationship between Sp1 and vascular endothelial growth factor (VEGF) expression, as well as their effect on survival in 86 cases of resected human gastric cancer. The degree of VEGF expression correlated highly with Sp1 expression ($P < 0.01$). Patients with high Sp1 expression were 98 times more likely to have high VEGF expression compared with those with negative Sp1 expression. Clinically, negative or weak Sp1 expression was associated with early stage (IA) in gastric cancer. Strong Sp1 expression was more frequently observed among patients with stage IB–IV disease ($P = 0.035$). Similarly, whereas strong Sp1 expression was uncommonly observed among patients with N0 or N1 disease (19 and 16%), N2/N3 gastric cancer was associated with strong Sp1 expression (48%; $P = 0.034$). Strong Sp1 expression was also associated with inferior survival. The median survival duration in patients who had a tumor with a negative, weak, and strong Sp1 expression was 44, 38, and 8 months ($P = 0.0075$), respectively, whereas patients with strong VEGF expression had a shorter survival duration; the difference was not statistically significant. When Sp1 and VEGF expression, stage, completeness of resection, histology, and patient age were entered in a Cox proportional hazards model, strong Sp1 expression ($P = 0.021$) and an advanced disease stage ($P < 0.001$) were independently prognostic of poor survival. Given the importance of Sp1 in the expression of VEGF, our data suggest that dysregulated Sp1 expression and activation play important roles in VEGF overexpression and, thus, gastric cancer development and progression.

INTRODUCTION

Although the incidence of gastric cancer declined in the West from the 1940s to the 1980s, it remains a major public health problem throughout the world (1). In Asia and parts of South America in particular, gastric cancer is the most common epithelial malignancy and leading cause of cancer-related deaths. In fact, gastric cancer remains the second most frequently diagnosed malignancy worldwide and cause of 12% of all cancer-related deaths each year (1, 2). Advances in the treatment of this disease are likely to come from a fuller understanding of its biology and behavior. The aggressive nature of human gastric carcinoma is related to mutations of various oncogenes and tumor suppressor genes (3–5) and abnormalities in several growth factors and their receptors (4, 5). These abnormalities affect the downstream signal transduction pathways involved in the control of cell growth and differentiation. Specifically, these perturbations confer a tremendous survival and growth advantage to gastric cancer cells.

The growth and metastasis of cancer depend on angiogenesis, which is the formation of new blood vessels from a preexisting network of capillaries (6). Of the numerous angiogenic factors discovered thus far, vascular endothelial growth factor (VEGF; Ref. 7), also known as vascular permeability factor (8), has been identified as a key mediator of tumor angiogenesis. Previous studies have demonstrated significantly elevated expression of VEGF in biopsy specimens of human gastric cancer (9–11). The mechanism of VEGF expression and its regulation in human gastric cancer are mostly unknown. Increasing evidence suggests that VEGF expression is regulated by a plethora of external factors. Major stimulators of VEGF expression include hypoxia and acidosis (12, 13), hormones, cytokines, and growth factors (14). Also, many tumor cells can constitutively express VEGF *in vitro* without any apparent external stimulation (15). In fact, loss or inactivation of tumor suppressor genes and activation of oncogenes are associated with VEGF overexpression (16, 17). VEGF promoter analyses have revealed several potential transcription factor-binding sites such as hypoxia-inducible factor 1, activator protein 1, activator protein 2, Egr-1, Sp1, and many others (18–22), suggesting that

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multiple signal transduction pathways may be involved in VEGF transcription regulation. A recent study by our group suggested that abnormal Sp1 activation augments the angiogenic and metastatic capacity of tumor cells through overexpression of multiple Sp1 downstream genes, including VEGF (15). Sp1 is a sequence-specific DNA-binding protein that is important in the transcription of many cellular and viral genes that contain GC boxes in their promoter (23). Additional transcription factors (Sp2, Sp3, and Sp4) similar to Sp1 in their structural and transcriptional properties have been cloned, thus forming a Sp1 multigene family (24–30). Aberrant activities of various members of the Sp1 family have been implicated in tumor development and progression (15, 31–33), although the signaling mechanisms remain unknown. Because VEGF and Sp1 markers predict patient survival, we asked whether the Sp1 expression levels predict the VEGF expression levels given that Sp1 has been implicated as critical to VEGF regulation.

In the present study, we examined the expression of and relationship between Sp1 and VEGF in the tissue of patients with resected gastric cancer and their impact on survival duration. We found that elevated Sp1 activation and concomitant VEGF overexpression occurred in patients with human gastric cancer and was inversely correlated with survival, suggesting that abnormally activated Sp1 expression represents a potential molecular marker for poor prognosis and directly contributes to gastric cancer development and progression.

MATERIALS AND METHODS

Human Tissue Specimens and Patient Information. We used human gastric cancer tissue specimens preserved in the Gastric Cancer Tissue Bank at and obtained information about the patients from The University of Texas M. D. Anderson Cancer Center Upper Gastrointestinal Carcinoma Database. Primary gastric cancer in these patients was diagnosed and treated at M. D. Anderson Cancer Center from 1985 to 1998. The patients had well-documented clinical histories and follow-up information. None of them underwent preoperative chemotherapy and/or radiation therapy. Eighty-six patients with primary gastric cancer were randomly selected for this study. All of the patients had undergone gastrectomy with lymph node dissection. Gastric cancer centered at or above the gastroesophageal junction was not included in this study. Proximal gastric cancer centered below the gastroesophageal junction was included. Gastroesophageal junction cases associated with Barrett's were not included.

Western Blot Analysis. Whole-cell lysates were prepared from human normal and gastric cancer tissue specimens. Four paired normal gastric and gastric tumor tissue specimens were selected from the patients with known expression levels of Sp1 and VEGF, as well as similar percentage of tumor epithelial cells present relative to stroma as confirmed by immunostaining. Standard Western blotting was performed using a polyclonal rabbit antibody against human Sp1 (clone PEP2; Santa Cruz Biotechnology, Santa Cruz, CA), a polyclonal rabbit antibody against human VEGF, and antirabbit IgG, a horseradish peroxidase-linked $F(ab')_2$ fragment obtained from a donkey (Amersham Life Sciences, Arlington Heights, IL). Equal protein sample loading was monitored by incubating the same membrane filter with an anti-β-actin antibody (15). The probe proteins were detected using the Amersham-enhanced chemiluminescence system according to the manufacturer's instructions.

Immunohistochemistry. Sections (5-μm thick) of formalin-fixed, paraffin-embedded tumor specimens were deparaffinized in xylene and rehydrated in graded alcohol. Antigen retrieval was performed with 0.05% saponin for 30 min at room temperature. Endogenous peroxidase was blocked using 3% hydrogen peroxide in PBS for 12 min. The specimens were incubated for 20 min at room temperature with a protein-blocking solution consisting of PBS (pH 7.5) containing 5% normal horse serum and 1% normal goat serum and then incubated at 4°C in a 1:200 dilution of rabbit polyclonal antibody against human Sp1 (clone PEP2) or 1:100 dilution of rabbit polyclonal antibody against human VEGF (Santa Cruz Biotechnology). The samples were then rinsed and incubated for 1 h at room temperature with peroxidase-conjugated antirabbit IgG. Next, the slides were rinsed with PBS and incubated for 5 min with 3,3′-diaminobenzidine (Research Genetics, Huntsville, AL). The sections were washed three times with distilled water, counterstained with Mayer's hematoxylin (BioGenex Laboratories, San Ramon, CA), and washed once each with distilled water and PBS. Afterward, the slides were mounted using a Universal Mount (Research Genetics) and examined using a bright-field microscope. A positive reaction was indicated by a reddish-brown precipitate in the nuclei (Sp1) or cytoplasm (VEGF; Refs. 34, 35). Depending on the percentage of positive cells and staining intensity, Sp1 and VEGF staining were classified into three groups: negative; weak positive; and strong positive. Specifically, the percentage of positive cells was divided into five grades (percentage scores): <10% (0), 10–25% (1), 26–50% (2), 51–75% (3), and >75% (4). The intensity of the staining was divided into four grades (intensity scores): no staining (0); light brown (1); brown (2); and dark brown (3). Sp1-staining positivity was determined using the following formula: overall score = percentage score × intensity score. An overall score of ≤3, > 3 to ≤6, and >6 was defined as negative, weak positive, and strong positive, respectively.

Statistical Analysis. The two-tailed χ^2 test was performed to determine the significance of the difference between the covariates. Survival durations were calculated using the Kaplan-Meier method. The log-rank test was used to compare cumulative survival in the patient groups. The Cox proportional hazards model was used to provide univariate and multivariate hazard ratios for the study parameters. Each patients' level of VEGF and Sp1 expression, age, sex, Lauren's classification, disease stage (American Joint Committee on Cancer), and completeness of surgical resection (R0 *versus* R1 and R2) were included in the model. In all of the tests, $P < 0.05$ was defined as statistically significant. The SPSS software program (version 11.05; SPSS Inc., Chicago, IL) was used in the analyses.

RESULTS

Patient Characteristics. Eighty-six cases were selected to represent all stages and histological types of malignant gastric cancer. The median duration of follow-up was 25.9 months. At the last follow-up examination, 27 patients were still alive, whereas 59 patients had died. Eighty-six primary tumor and 53

Table 1 Patient characteristics and VEGF expression in 86 patients with resected gastric cancer[a]

Parameter	All	VEGF[b], *n*		
		Negative	Weak	Strong
Gender				
Men	56	12	30	14
Women	30	2	19	9
Race/ethnicity				
Asian	11	2	9	0
Black	11	2	3	6
Hispanic	14	2	8	4
Non-Hispanic white	50	8	29	13
Age (yrs)				
Mean (SD)	62 (14)	55 (14)	63 (13)	66 (16)
Tumor location				
Proximal	20	2	10	8
Mid	7	0	4	2
Distal	54	11	31	12
Diffuse	3	0	2	1
Remnant	2	2	1	0
Stage				
I	14	3	10	1
II	28	3	17	7
III	30	6	14	10
IV	14	2	7	5
Residual disease				
R0	69	10	43	16
R1/R2	17	4	6	7
Gastrectomy				
Partial	54	12	30	12
Complete	32	2	19	11
Lymphadenectomy				
D0	1	0	1	8
D1	36	6	22	8
D2	35	8	17	10
Unknown	14	3	8	3
Lauren's histology				
Intestinal	57	16	36	15
Diffuse	29	8	13	8
WHO grade				
1–2	35	3	27	5
3–4	51	11	22	18

[a] No statistically significant differences in the patient characteristics according to Sp1 group were observed.
[b] VEGF, vascular endothelial growth factor.

lymph node metastasis samples were examined. There were 56 men and 30 women, and their mean age was 62 years. Proximal cancer localization was observed in 20 cases. The histology was intestinal in 57 cases and diffuse in 29 cases. Details of the patient characteristics are provided in Table 1.

Sp1 and VEGF Expression in Human Normal Gastric and Tumor Tissue. Sp1 and VEGF expression were evaluated in the primary cancer tissue of 86 patients via immunohistochemistry. Strong Sp1 expression was observed in 21 (24%) cases; Sp1 expression was classified as weak and negative in 48 (56%) and 17 (20%) cases, respectively. VEGF was strongly expressed in 23 (27%) cases; weak and negative VEGF expressions were observed in 49 (57%) and 14 (16%) cases, respectively (Table 1). No significant differences in the distribution of gender, race/ethnicity, tumor location, type of resection, residual disease status, extent of lymphadenectomy, and Lauren's classification were detected among the three VEGF expression categories. There was a trend toward older age among patients with strong Sp1 expression. VEGF expression was highly correlated with Sp1 expression (Table 2; $P < 0.0001$).

To technically validate the immunostaining data, we also performed Western blot analysis using selected paired samples of gastric tumor and adjacent normal tissue with known Sp1 and VEGF expression detected via immunostaining. Clearly, the protein levels in the Western blot analysis were consistent with immunohistochemical staining scores (Fig. 1). By analyzing consecutive sections, we found that both Sp1 and VEGF protein was predominantly localized in tumor epithelial cells, whereas little was detected in stroma, and the Sp1 expression pattern was consistent with that of VEGF (Fig. 2). Moreover, the levels of VEGF protein expression also were consistent with those of Sp1 (Fig. 3). These data suggested that aberrant Sp1 activation may have caused the VEGF overexpression in tumor cells.

Association between Sp1 and VEGF expression. To further test the association between Sp1 and VEGF expression, we performed ordinal logistic regression analysis in which VEGF was the response variable with three levels (coded 0 for negative, 1 for weak, and 2 for strong). To evaluate whether the

Table 2 Sp1 versus VEGF expression in primary gastric cancer[a]

	VEGF[b] expression		
	Negative	Weak	Strong
Sp1 expression			
Negative	8	8	1
Weak	5	37	6
Strong	1	4	16

[a] Level of VEGF expression correlates with level of Sp1 expression ($P < 0.001$).
[b] VEGF, vascular endothelial growth factor.

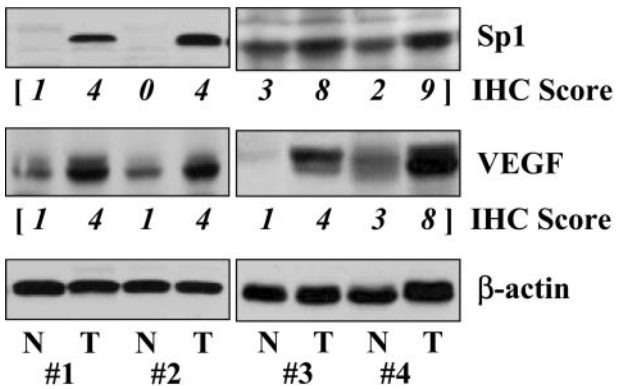

Fig. 1 Sp1 and vascular endothelial growth factor (VEGF) protein expression in human gastric cancer tissue. Whole-cell protein extracts were prepared from four paired normal gastric (N) and gastric tumor tissue (T) specimens obtained from the patients with Sp1 and VEGF expression detected via immunostaining [immunohistochemical (IHC) scores in *italics*]. The level of expression of Sp1 and VEGF protein was determined using Western blot analysis with 10-μg whole-cell protein extracts. Equal protein sample loading was monitored by hybridizing the same membrane filter with an anti-β-actin antibody. Of note is that the levels of both Sp1 and VEGF protein expression were significantly elevated in tumor tissue compared with those in normal tissue.

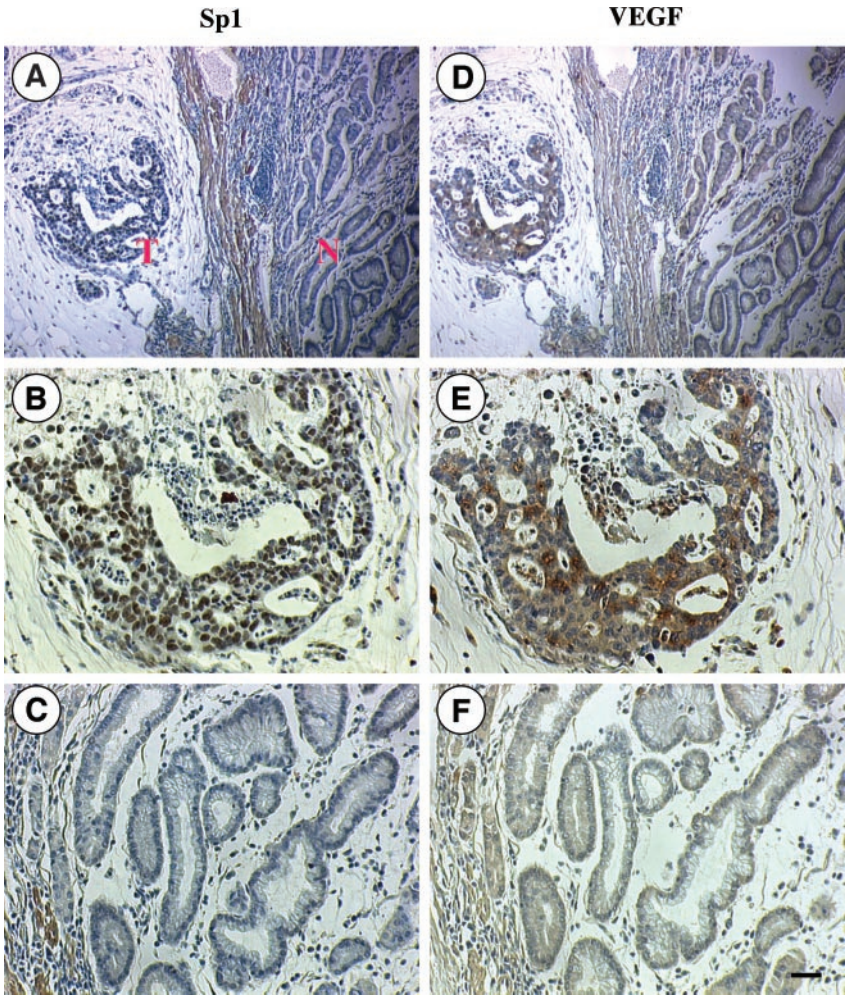

Fig. 2 Colocalization of Sp1 and vascular endothelial growth factor (VEGF) protein expression in human gastric cancer tissue and adjacent normal tissue. Tissue sections were prepared from formalin-fixed, paraffin-embedded specimens of gastric tumors. Immunohistochemical staining was performed using specific anti-Sp1 and –anti-VEGF antibodies. Of note is that compared with adjacent normal tissue cells (N), the majority of the tumor cells (T) were strongly positive for Sp1 in the nuclei, a pattern that was similar to that of VEGF expression. *B* and *C* are representative areas from *A*, whereas *E* and *F* are representative areas from *B* with higher magnification. *Calibration bar*, 50 μm (*A* and *D*) and 20 μm (*B*, *C*, *E*, and *F*).

model was constrained by proportional odds assumption, a score statistic test was estimated and shown to be not statistically significant ($P = 0.199$), which proved that the model assumption holds. Using this model, we estimated that the odds ratio = exp (β), whereas the 95% confidence interval = exp [β ± 1.96 (SE)]. Table 3 shows that patients with gastric cancer who had weak and strong Sp1 expression were 6 and 98 times more likely to have a high level of VEGF expression, respectively, when compared with those with negative Sp1 expression. Grouping mild and moderate expression of Sp1 into one category *versus* high expression did not change the results. The estimated odds ratio was 22.8 (95% confidence interval, 6.63–78.01).

Sp1 Expression and Stage. We hypothesized that increased Sp1 expression may be an early event leading to increased invasiveness and metastatic potential. Thus, we compared the Sp1 expression pattern in earliest stage (IA) gastric cancer with that in more advanced gastric cancer (stages IB–IV). Early gastric cancer was associated with negative and weak Sp1 expression (Table 4), whereas more advanced disease was associated with strong Sp1 expression. Among patients with stage IA gastric cancer, 57 and 29% had negative and weak Sp1 expression, respectively. Strong Sp1 expression was observed in only 14% of early gastric cancer cases. Among patients with stage IB–IV disease, negative Sp1 expression was uncommon (15%), whereas weak and strong Sp1 expression was observed in 58 and 25% of the cases, respectively. The difference in expression was statistically significant ($P = 0.035$).

It is also known that patients with a high number of nodal metastases (N2/N3) have a particularly poor prognosis. Therefore, we compared the Sp1 expression pattern in patients with N0, N1, and N2/N3 (more than six nodal metastases) disease. In this comparison, N2/N3 disease was associated with strong Sp1 expression ($P = 0.034$). Among patients with N0 and N1 disease, 19 and 16% had strong Sp1 expression, respectively, whereas 48% of the patients with N2/N3 disease had strong Sp1 expression.

Effects of Sp1 and VEGF Expression on Patient Survival. The median survival duration in patients who had a tumor with a negative, weak, and strong Sp1 expression was 44, 38, and 8 months, respectively. Elevated Sp1 expression was associated with an inferior survival duration ($P = 0.0075$; Table 5). A trend toward inferior survival in patients with strong VEGF expression was observed. The median survival duration

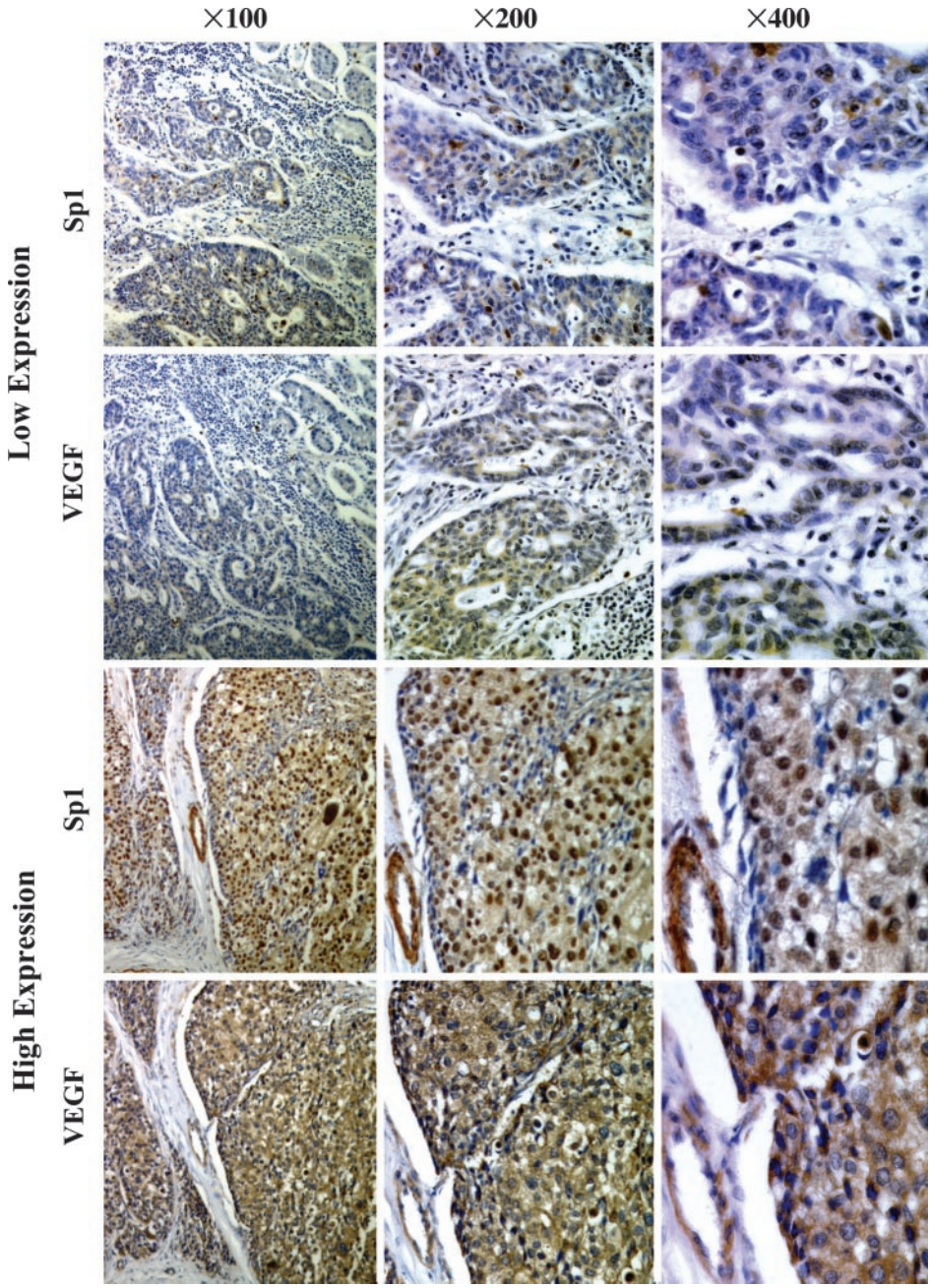

Fig. 3 Differential Sp1 and vascular endothelial growth factor (VEGF) expression in human gastric cancer tissues. Immunohistochemical staining was performed on one pair of gastric tumor specimens representing negative/low and high expression levels of Sp1 and VEGF. Pictures of representative areas were presented at different magnifications (×100, ×200, and ×400).

in patients who had a tumor with a negative, weak, and strong VEGF expression was 29, 41, and 14 months, respectively. The impact of VEGF expression on survival duration was not statistically significant, however. Other parameters that affected survival in univariate analyses included American Joint Committee on Cancer stage ($P < 0.0001$) and completeness of resection ($P < 0.0002$). The patients' age at diagnosis (as a continuous variable in univariate Cox proportional hazards analysis), histological grade, and Lauren's classification did not have a statistically significant effect on survival.

Next, the patients' level of Sp1 and VEGF expression, American Joint Committee on Cancer stage, completeness of resection, age, grade, and histology were entered in a Cox proportional hazards model for multivariate analysis (Table 6). Adjusting for the effect of covariates, strong Sp1 expression ($P = 0.022$) and an advanced stage ($P < 0.001$) were independent predictors of poor survival. The prognosis of patients with strong Sp1 expression is significantly worse (hazard ratio, 3.6; 95% confidence interval, 1.2–10.6) than those with negative Sp1 expression, which was significantly higher than that for the group with negative Sp1 expression (referent). In multivariate analyses, VEGF expression did not significantly affect survival.

Table 3 ORs of strong VEGF expression by Sp1 status[a]

Sp1 expression	OR (95% CI)[b]	*P*
Negative	1.0 (referent)	
Weak	5.6 (1.6–19.0)	0.0060
Strong	98.5 (18.9–511.0)	0.0001

[a] Odds of high VEGF expression and weak and strong Sp1 expression group compared with the negative Sp1 expression group.
[b] VEGF, vascular endothelial growth factor; OR, odds ratio; CI, confidence interval.

The hazard ratio in the group with weak and strong VEGF expression was 0.89 and 0.87, respectively. A trend toward improved survival ($P = 0.093$) was observed in patients who had a complete resection (R0). The patients' age at diagnosis, histological grade, and Lauren's classification did not significantly affect survival in the multivariate analyses.

DISCUSSION

Herein we offer direct clinical evidence of a strong correlation between Sp1 and VEGF expression using immunohistochemistry with confirmation via Western blot analysis. Although both VEGF and Sp1 expression predicted poor patient survival, Sp1 is clearly a better predictor. We also provided evidence of the role of Sp1 in tumor invasion and metastasis in that higher levels of Sp1 expression were associated with disease progression to a more advanced stage when the cases were divided into early- and late-stage groups. Similarly, Sp1 expression was associated with more advanced disease when the cases were sorted by number of nodal metastases. Our study indicated that Sp1 is an independent prognostic marker for human gastric cancer and that dysregulated expression of it may influence many aspects of gastric cancer biology, including VEGF overexpression. Therefore, targeting the Sp1 signaling pathway may be used to control gastric cancer development and progression.

Molecular markers of poor prognosis include fibroblast growth factor receptor 1 (36), epidermal growth factor receptor (37–40), insulin-like growth factor receptor (41), hepatocyte growth factor receptor (42–44), insulin-like growth factor binding protein 2 (45, 46), matrix metalloproteinase 2 (47–49), and urokinase plasminogen activator (50, 51). VEGF (10, 11, 15, 52–55) and basic fibroblast growth factor (56), which are key angiogenic factors, have also been indicated to be independent prognostic factors for gastric cancer. In searching for better novel prognostic factors, we recently found that the transcription factor Sp1 is a prognostic marker (33). Because Sp1 is an essential transcription factor for many genes that may regulate many aspects of cancer biology, including survival, growth, invasion, and angiogenesis, abnormal Sp1 expression and activation may be a more powerful predictor of patient outcome. In the present study, multivariate survival analyses showed that the Sp1 expression level and disease stage were the only independent predictors of outcome. The VEGF expression level, on the other hand, did not have a statistically significant effect on survival. Given its central role in regulating multiple genes that lead to aggressive growth and metastasis, Sp1 is a better predictor of clinical outcome than VEGF. This strengthens our hypothesis that Sp1 controls multiple pathways that mediate the aggressive behavior of gastric cancer. In fact, the role of Sp1 in tumor invasion and metastasis was evidenced by our findings in that higher levels of Sp1 expression were associated with progression to higher stage disease. Similarly, Sp1 expression was associated with more advanced disease when the cases were divided into groups with a low and high number of nodal metastases. However, we did not observe a statistically significant difference in Sp1 expression when the cases were divided into the six American Joint Committee on Cancer stage groups. This was likely because of the limitation of our sample size.

Although gene expression analyses have suggested a regulatory role for Sp1 in VEGF expression, it is unknown whether overexpression of VEGF is caused by aberrant Sp1 expression and activation. In the present study, we found direct clinical evidence of a strong correlation between Sp1 and VEGF ex-

Table 4 Association of Sp1 expression with a high number of metastatic lymph nodes and advanced disease stage

	Sp1 expression, *n* (%)			
	Negative	Weak	Strong	*P*
Stage[a]				
IA	4 (57)	2 (29)	1 (14)	0.035
II–IV	13 (17)	46 (58)	20 (25)	
N[b]				
0	4 (15)	18 (67)	5 (19)	0.034
1	11 (29)	21 (55)	6 (16)	
2+	2 (9)	9 (43)	10 (48)	

[a] Stage IA, $T_1N_0M_0$.
[b] N0, 0 nodes involved; N1, 1–6 nodes involved; N2, 7–15 nodes involved; N3, >15 nodes involved.

Table 5 Univariate survival analyses

Parameter	Median survival duration (mo)	*P*
Sp1 expression		
Negative	44	0.0075
Weak	38	
Strong	8	
VEGF[a] expression		
Negative	29	0.074
Weak	41	
Strong	14	
AJCC stage		
I	91	<0.001
II	64	
III	23	
IV	9	
Resection status		
R0	41	0.0002
R1 and R2	11	
Lauren's histology type		
Intestinal	41	0.0605
Diffuse	15	
Age (continuous variable)[b]	HR 1.004	0.701
WHO grade		
1–2	40	0.3469
3–4	22	

[a] VEGF, vascular endothelial growth factor; AJCC, American Joint Committee on Cancer; HR, hazard ratio.
[b] Estimated using the Cox proportional hazards model.

Table 6 Multivariate survival analyses

Parameter	Multivariate HR[a,b] (95% CI)	*P*
Sp1 expression		
Negative	1.00 (referent)	
Weak	2.07 (0.90–4.77)	0.087
Strong	3.54 (1.20–10.42)	0.022
VEGF expression		
Negative	1.00 (referent)	
Weak	0.86 (0.37–1.99)	0.72
Strong	0.88 (0.32–2.46)	0.81
AJCC stage		
I	1.00 (referent)	
II	1.57 (0.53–4.65)	0.416
III	5.97 (2.10–16.98)	0.001
IV	7.55 (2.11–27.02)	0.002
Resection status		
R0	1.00 (referent)	
R1 and R2	2.11 (0.88–5.05)	0.093
Lauren's classification		
Intestinal	1.00 (referent)	
Diffuse	0.78 (0.36–1.68)	0.526
WHO grade		
1–2	1.00 (referent)	
3–4	0.67 (0.39–1.84)	0.668
Age (continuous variable)	1.00 (0.97–1.02)	0.843

[a] HRs estimated using multivariate Cox proportional hazards modeling.

[b] HR, hazard ratio; CI, confidence interval; VEGF, vascular endothelial growth factor; AJCC, American Joint Committee on Cancer.

pression corroborating previous laboratory findings (15). Additional analyses of other downstream molecules in which the expression is regulated by Sp1 are in progress. In addition, Sp1 may be a useful molecular marker for selecting patients with a poor prognosis to receive more aggressive preoperative or adjuvant therapy in clinical trials. Gastric cancer is a disease in which multiple growth factor pathways are involved though multiple tyrosine kinases such as epidermal growth factor receptor, HER2, VEGFR1, VEGFR2, platelet-derived growth factor receptor, and c-met. Inhibition of one such pathway may not be sufficient for antitumor activity. Sp1, with its central regulatory role in many such pathways, is an attractive target for therapeutic development. However, additional studies are clearly needed to test whether Sp1 is a reliable tumor progression marker and/or effective therapeutic target for gastric cancer.

On the other hand, the underlying mechanisms that result in Sp1 overactivation are currently unknown. During tumor development and progression, Sp1 can be overactivated through several potential mechanisms, including genetic and epigenetic pathways (23, 28–30, 57–66). Whether oncogenes and/or tumor suppressor genes contribute to constitutive Sp1 activation in human cancer cells and how their status affects that activation are not fully elucidated. It appears that activated oncogenes, including Ras, Src, and Raf, enhance the transcription activity of Sp1 through constitutive activation of the p42/p44 MAPK pathway, which has been observed often in many human tumors. In fact, p42/p44 MAPK directly phosphorylates Sp1 on threonines 453 and 739 and increases the DNA-binding ability of Sp1, thus activating many Sp1-regulated genes implicated in tumor growth and progression (66, 67).

Other factors may also be involved in Sp1 overactivation, *e.g.*, a stressful tumor microenvironment in advanced gastric cancer. It has been shown that Sp1 activity can be modulated by hypoxia (68, 69) and overproduction of free radicals, *e.g.*, reactive oxygen species and nitric oxide (70–72), which are prominent stress factors in the tumor microenvironment. Several lines of evidence have additionally indicated that such stress factors can activate the p42/p42 MAPK pathway and, to a lesser extent, c-Jun NH_2-terminal kinase-related signaling pathways, which may be responsible for Sp1 overactivation (66). Therefore, both genetic and epigenetic factors can cause Sp1 overactivation, which leads to altered expression of multiple Sp1 downstream genes and contributes to tumor growth and metastasis.

In summary, we found that the level of Sp1 expression was directly related to the level of VEGF expression, which is closely related to the postoperative prognosis for gastric cancer. Preoperative determination of Sp1 activity may be useful in deciding on the modality for and determining the extent of postoperative therapy. However, biological and experimental evidence is clearly required to demonstrate the causal role of Sp1 in VEGF regulation in and development and progression of human gastric cancer.

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