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Original Article

STAT3 regulation the expression of VEGF-D in HGC-27 gastric cancer cell

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Abstract: Objective: To explore the potential mechanism of vascular endothelial growth factor D (VEGF-D) contribution to the lymphangiogenesis was regulated by the signal transducer and activator of transcription 3 (STAT3). Methods: We detected the expression in GC tissue, adjacent non-tumor tissue, GC cell lines (AGS, SUN-1, KATO-III, BGC-823, MGC-803, SGC-7901, and HGC-27), and GES-1 cell line. STAT3 siRNA transfection and genome microarray were applied to demonstrate whether the expression of VEGF-D was mediated by the STAT3 in GC. Results: We showed the STAT3, pSTAT3, and VEGF-D expression in GC tissue was significantly higher than those in adjacent non-tumor tissue, respectively. In addition, both STAT3 and VEGF-D mRNA expression was much higher in each GC cell line than those in GES-1 cell line. With STAT3 siRNA transfection, we demonstrated that VEGF-D expression level decreased significantly in HGC-27 cell by using the genome microarray representing STAT3 potential regulation the VEGF-D expression. Conclusion: STAT3, a novel signal transducer inactivating in the GC cell, can contribute to the lymph node metastasis by promoting lymphangiogenesis via up-regulation expression of VEGF-D.

Keywords: Stomach, neoplasm, signal transducer and activator of transcription 3, vascular endothelial growth factor, lymphatic metastasis

Introduction

Lymphatic metastasis is an important approach for cancer cell dissemination in various solid malignancies. In theory, lymphangiogenesis is a necessary prerequisite for the lymph node metastasis from gastric cancer (GC) [1]. Subsequently, lymphangiogenesis was identified to be significantly associated with the prognosis of GC [2-4]. As the critical endogenous molecules inducing the lymphangiogenesis, vascular endothelial growth factor (VEGF) -C and VEGF-D were negatively relative to the overall survival (OS) of GC patients [5-8]. Signal transducer and activator of transcription 3 (STAT3), a poor survival biomarker potential association with lymph node metastasis, was identified to be a negative prognostic predictor of GC patients after curative resection in our previous study [9]. Recently, several researchers demonstrated the expression of the STAT3 was potentially associated with the expression of VEGF-C in various malignant diseases, which indicated that the STAT3 might be an active participant in lymphangiogenesis and lymph node metastasis [10-14]. It is still unclear whether VEGF-D, an important special lymphangiogenesis factor demonstrated in other cancers, can be regulated by STAT3 in GC.

In view of the obviously negative prognostic affection of lymph node metastasis, we intend to elucidate the detailed molecular mechanism of lymphatic dissemination via STAT3 signaling pathway in GC. Therefore, we preliminarily designed this study to explored the expression of the lymphangiogenesis factor (VEGF-C and VEGF-D) in both cell line and tissue, and then demonstrated the VEGF-D was mediated by the STAT3 in GC.

Materials and methods

Cell lines

Human GC cell lines (AGS, SUN-1, KATO-III, BGC-823, MGC-803, SGC-7901, and HGC-27) were purchased from the Type Culture Collection

Table 1. Patient demographics

Variables	Case (%)
Age at surgery (year)	
≤ 55	40 (37.4%)
> 55	67 (62.6%)
Gender	
Male	71 (66.4%)
Female	36 (33.6%)
Size of tumor	
< 4 cm	15 (14.0%)
≥ 4 cm	92 (86.0%)
Location of tumor	
Upper third	21 (19.6%)
Middle third	36 (33.6%)
Lower third	43 (40.2%)
More than 2/3 stomach	7 (6.5%)
Lauren's classification	
Intestinal	30 (28.0%)
Diffuse	74 (69.2%)
Mixed	3 (2.8%)
N stage (According to the 7th edition TNM classification for GC)	
N0	31 (29.0%)
N1	18 (16.8%)
N2	20 (18.7%)
N3	38 (35.5%)
Extent of metastatic lymph nodes	
No	31 (29.0%)
Perigastric	24 (22.4%)
Extragastric	52 (48.6%)
T stage (According to the 7th edition TNM classification for GC)	
T1	5 (4.5%)
T2	8 (7.5%)
T3	6 (5.6%)
T4	87 (82.4%)
STAT-3 expression in GC tissue	
Positive	67 (62.6%)
Negative	40 (37.4%)
STAT-3 expression in adjacent non-tumor tissue	
Positive	9 (8.4%)
Negative	98 (91.6%)
pSTAT-3 expression in GC tissue	
Positive	41 (38.3%)
Negative	66 (61.7%)
pSTAT-3 expression in adjacent non-tumor tissue	
Positive	2 (1.9%)
Negative	105 (98.1%)
VEGF-C expression in GC tissue	
Positive	41 (38.3%)

of the Chinese Academy of Sciences, (Shanghai, China). Human normal gastric mucosa cell GES-1 line was purchased from Biowit Technologies Corporation (Shenzhen, China). All GC cell lines and GES-1 cell lines were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in RPMI 1640 (Thermo Electron Corporation, Beijing, China). Media were supplemented with 10% (v/v) FBS (Life Tech, Mulgrave Vic, Australia) and penicillin/streptomycin (10,000 IU/ml penicillin, 20 mg/ml streptomycin; Roche, Swiss). The medium was changed twice a week.

Tissue samples

After the curative gastrectomy, 107 gastric adenocarcinoma tissues, and 107 matched adjacent non-tumor tissues were retrieved from the Department of Gastric Cancer Surgery, Tianjin Medical University Cancer Hospital (Tianjin, China). The research protocol was approved by the Research Ethics Committee of Tianjin Medical University Cancer Institute and Hospital (registration number: ChiCTR-TRC-10000785), and written informed consent was obtained from all patients (Tianjin, China). All surgical specimens were collected between January 2004 and September 2007 at Tianjin Medical University Cancer Hospital (Tianjin, China). According to the 7th edition TNM classification for GC, 24 patients (22.4%) were pathologically identified to present with the perigastric lymph node metastasis, whereas 52 patients (48.6%) presented with extragastric lymph node metastasis after surgery. The mean number of the dissected lymph nodes was 24.47 ± 9.84 (range: 15-61), and the mean number of the metastatic lymph nodes was 6.81 ± 4.84 (range: 0-42). Until the follow-up was over, 87 patients (81.3%) included in this study were dead. In addition, no patient died during the initial hospital stay or for 1 month after surgery. The clinical data and patient characteristics are shown in **Table 1**.

Negative	66 (61.7%)
VEGF-C expression in adjacent non-tumor tissue	
Positive	22 (20.6%)
Negative	85 (79.4%)
VEGF-D expression in GC tissue	
Positive	47 (43.9%)
Negative	60 (56.1%)
VEGF-D expression in adjacent non-tumor tissue	
Positive	15 (14.0%)
Negative	92 (86.0%)

Surgical procedure

Curative resection was defined as a complete lack of grossly visible tumor tissue and metastatic lymph nodes remaining after resection, with pathologically negative resection margins. Primary tumors were resected en bloc with the extended lymphadenectomy.

Immunohistochemistry

Paraffin sections (4 μm thick) were deparaffinized and rehydrated. Antigen retrieval treatment was done at 95°C for 40 minutes in 0.01 mol/L sodium citrate buffer (pH 6.0), and endogenous peroxidases were blocked using 3% hydrogen peroxide for 30 minutes. The primary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa, CA, USA; STAT3 (sc-7179) at 1:150 dilution, pSTAT3 (sc-8001-R) at 1:150 dilution, VEGF-C (sc-9047) at 1:200 dilution, and VEGF-D (sc-13085) at 1:100 dilution). All sections were incubated overnight with the primary antibody at 4°C. The sections were then treated with peroxidase using the labeled polymer method with Biosynthesis Peroxidase (Beijing, China) for 30 minutes. For immunostaining, the slides were heated in an auto clave at 140°C for 3 min for STAT3, pSTAT3, VEGF-C, and VEGF-D in 0.01 M citric acid buffer following deparaffinization for antigen retrieval. Antibody binding was visualized using the Avidin Biotin Complex (ABC) Elite Kit and 3, 3-diaminobenzine according to the manufacturer's instructions (City Key Laboratory of Tianjin Cancer Center, China). Sections were then counterstained in hematoxylin. Brest cancer and colon cancer specimens that were prepared, and then confirmed to be immunoreactive for the relevant antigens in preliminary studies, were used as positive controls for STAT3, pSTAT3, VEGF-C and VEGF-D. For general negative controls, the primary antibody was replaced with PBS. The percentage of positive cells for STAT3, VEGF-C, and VEGF-D was classified as 0 (none), 1 (< 10%), 2 (11-50%), and 3 (> 50%). The immunointensity for STAT3, VEGF-C, and VEGF-D was classified as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The total score of cell was obtained by adding the immunostaining score and the immunointensity score (range, 0-9). Scores from 2 to 9 were regarded as positive, whereas scores from 0 to 1 were regarded as negative. Positive staining for pSTAT3 expression was defined as > 25% nuclear staining with more than a moderate intensity for the tumor cell. The immunohistochemical expression was independently reviewed by two of the authors (J. D. and J. X).

Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR)

For the STAT3, VEGF-C and VEGF-D semi-quantitative RT-PCR, RNA was extracted from GC tissue, non-tumor adjacent tissue, seven GC cell lines, and GES-1 cell line using Trizol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. Total RNA was reverse transcribed to cDNA in a 20 ul volume using Reverse Transcription kit (Invitrogen, Carlsbad, CA, USA). Primers designed and utilized for STAT3, VEGF-C and VEGF-D were as follows: (forward/reverse sequences) STAT-3 (5'-CATTCGGAAAGTATTGTCGGC/ACATCGGCAGG TCAATGGTAT-3'), VEGF-C (5'-GCCCCAAACCAGTAACAATCA/CAGCATCC GAGGAAAACATAAA-3') and VEGF-D (5'-CTGCCTGATGTCAACTGCTTAG/AG ATGATCGCTTCACTGGTCC-3'). The GAPDH gene was used as an endogenous control for quantitative DNA-PCR. Primers designed and utilized for GAPDH was (forward/reverse sequences) (5'-TGGGTGTGAACCATGAGAAGT/TGAGTCCTTC CACGATACCAA-3'). The PCR Cycling conditions for all sequences were 35 cycles of denaturation at 95°C for 3 minutes, annealing for 30 seconds, and extension at 56°C for 30 seconds followed by a final extension at 72°C for 8 minutes. Annealing was performed at 72°C for STAT3, VEGF-C and VEGF-D. All PCR product electrophoreses were performed on a 2% agarose gel with ethidium bromide and visualized using the Gel Imager system (Asia Xingtai Mechanical

Table 2. Survival analysis of 107 gastric cancer patients

Variables	Median OS (months)	x^2 value	Univariate *p* value	Hazard ratio value	Multivariate *p* value
Age at surgery (year)					
≤ 55	21	0.001	0.969		
> 55	22				
Gender					
Male	21	5.763	0.016		
Female	14				
Size of tumor					
< 4 cm	25	1.201	0.273		
≥ 4 cm	20				
Location of tumor					
Upper third	18	4.946	0.176		
Middle third	20				
Lower third	26				
More than 2/3 stomach	21				
Lauren's classification					
Intestinal	21	0.226	0.893		
Diffuse	21				
Mixed	17				
N stage (According to the 7th edition TNM classification for GC)					
N0	42	33.996	< 0.001	1.570	< 0.001
N1	21				
N2	20				
N3	11				
Extent of metastatic lymph nodes					
No	42	22.659	< 0.001		
Perigastric	15				
Extragastric	17				
T stage (According to the 7th edition TNM classification for GC)					
T1	37	4.930	0.177		
T2	25				
T3	20				
T4	17				
STAT-3 expression in GC tissue					
Positive	18	10.214	< 0.001		
Negative	32				
pSTAT-3 expression in GC tissue					
Positive	15	18.451	< 0.001	0.431	0.001
Negative	36				
VEGF-C expression in GC tissue					
Positive	15	5.904	0.015		
Negative	22				
VEGF-D expression in GC tissue					
Positive	12	31.588	< 0.001		
Negative	34				

and Electrical Equipment Company, Beijing, China). The mRNAs were quantified by computerizing the gray scale values with Gel Imager system (Asia Xingtai Mechanical and Electrical

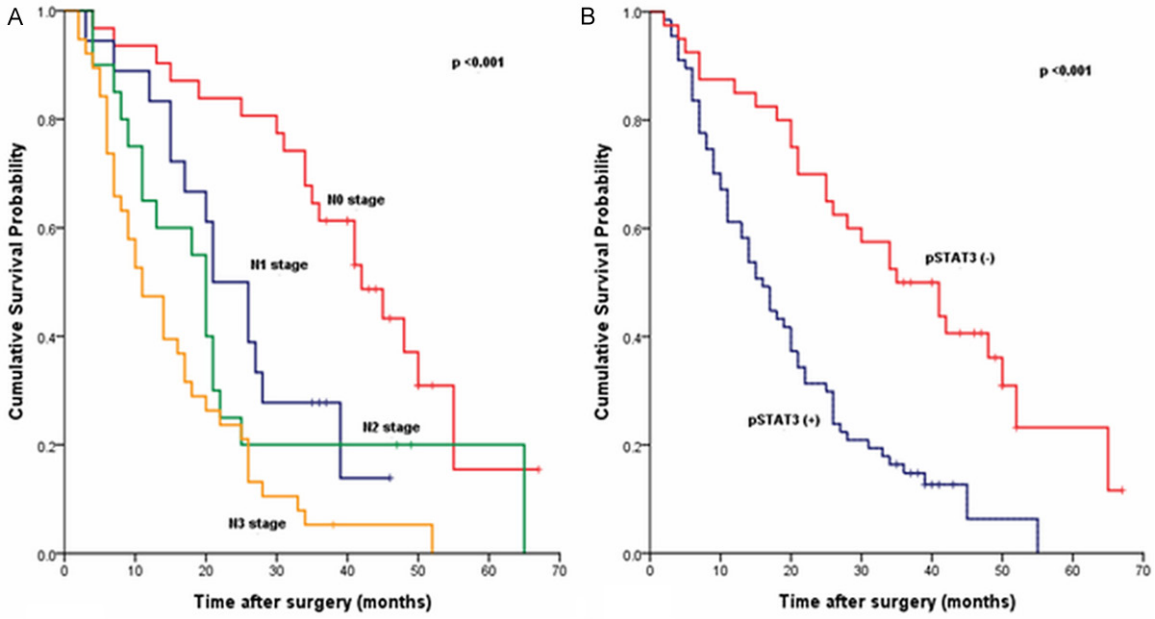

Figure 1. A. Survival curve for 107 GC patients following curative resection according to stage subgroup N stage (N0, N1, N2, or N3); B. Survival curve for 107 GC patients following curative resection according to stage subgroup pSTAT-3 protein expression (positive, or negative).

Equipment Co., Beijing, China). The relative expression values of STAT3, VEGF-C and VEGF-D mRNA were expressed by ratio between target mRNA gray scale value and GAPDH gray scale value.

Western blotting analysis

Cell lines were respectively added to 1 mL of 100 mmol/L Tris/HCl (pH 7.5), 100 mmol/L NaCl, 0.5% sodium deoxycholate, 1 mmol/L ethylenediaminetetraacetic acid, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate, and protease inhibitor. Protein (50 ug) per lane was then resolved using a 4% to 12% bis-Tris gel (Invitrogen) and transferred onto a nitrocellulose membrane (Invitrogen). The membrane was blocked for 1 h using Starting Block buffer (Pierce Biotechnology) and incubated at 4°C overnight with a primary antibody followed by incubation for 1 hat room temperature with fluorescence-labeled secondary antibodies (Molecular Probes). The following antibodies were used for Western blots: STAT3 (1:500; Santa Cruz Biotechnology), pSTAT3 (1:500; Santa Cruz Biotechnology), VEGF-C (1:1000; Santa Cruz Biotechnology) and VEGF-D (1:500; Santa Cruz Biotechnology). Gel Imager system (Asia Xingtai Mechanical and Electrical Equipment Company, Beijing, China) to analyze images and to determine gray values.

Short interfering RNA transfection

The short interfering RNA (siRNA) sequence used for targeting human STAT3 was synthesized by Agilent Technologies (sense/antisense: 5'-CCGGGCTGACCAAC AATCCCAAGAA CTCGAGTTCTTGGGATTGTTGGTCAGCTTTTT/ AATTAAAAAGCTGACCAACAATCCCAAGAA-CTCGAGTTCTTGGGATTGTTGGTCAGC-3'). A non-targeting siRNA pool was used as a negative control. siRNA transfections were performed with Lipofectamine 2000 (Invitrogen) reagent according to the manufacturer's protocol. HGC-27 cell was transfected with either 3 nmol/L siRNA against STAT3 or control siRNA during a period of 2 day. Two days after the first transfection, the cells were seeded for Western blotting analysis.

RNA microarray analysis

RNA purity and concentration were confirmed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA microarrays were manufactured by Agilent Technologies and contained 13067 human genes. Labeling and hybridization of total RNA samples (100 ng) were performed according to the manufacturer's protocol. The arrays were scanned with an Agilent microarray scanner (Agilent Tech-

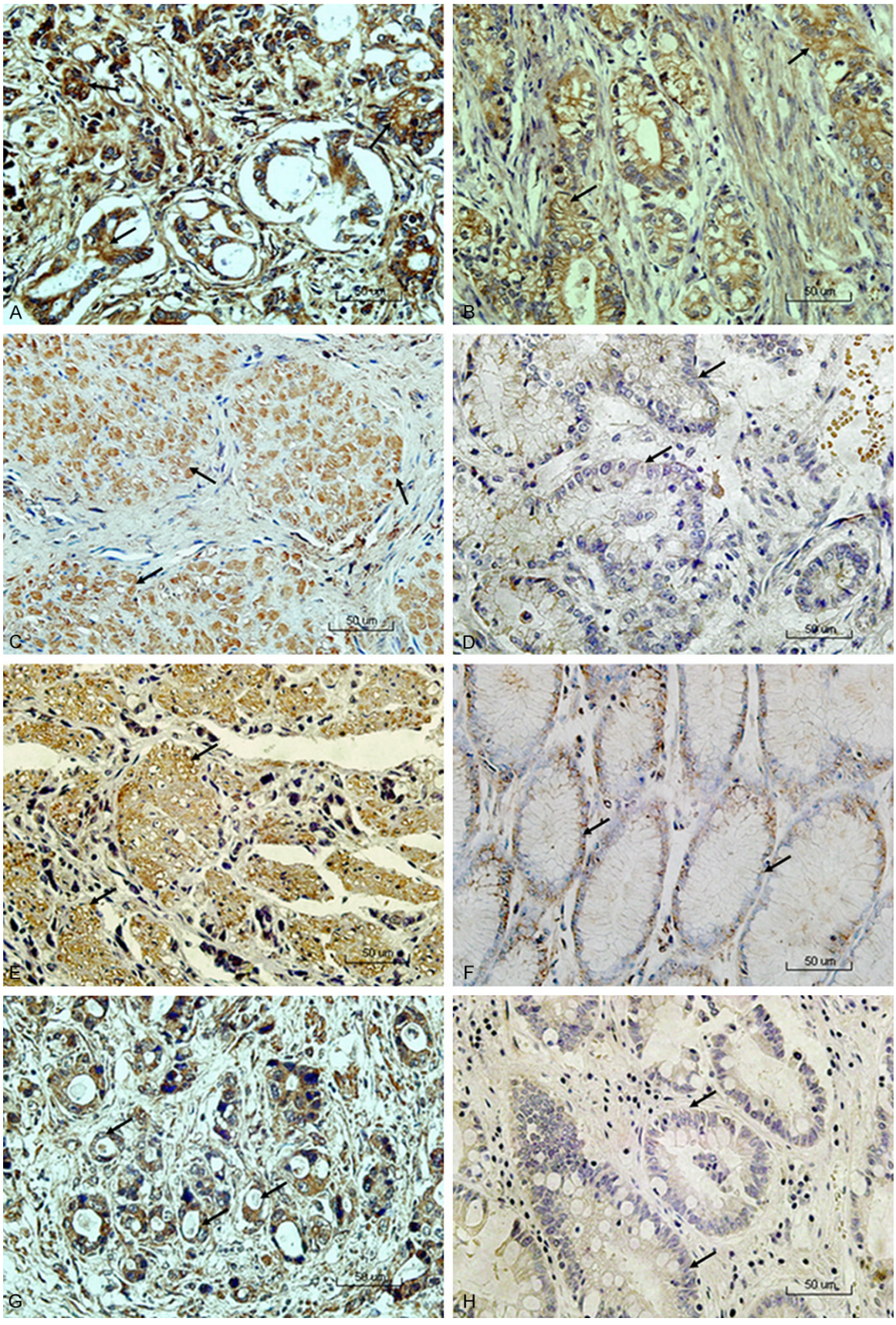
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Figure 2. A. Expression of STAT3 in the cytoplasm of malignant epithelium of GC tissue; B. Expression of STAT3 in the cytoplasm of epithelium of adjacent non-tumor tissue; C. Expression of pSTAT3 in the nucleus of malignant epithelium of GC tissue; D. Expression of pSTAT3 in the nucleus of epithelium of adjacent non-tumor tissue; E. Expression of VEGF-C in the cytoplasm of malignant epithelium of GC tissue; F. Expression of VEGF-C in the cytoplasm of epithelium of adjacent non-tumor tissue; G. Expression of VEGF-D in the cytoplasm of malignant epithelium of GC tissue; H. Expression of VEGF-D in the cytoplasm of epithelium of adjacent non-tumor tissue. All figures are at x400 magnification.

nologies) using high dynamic range settings as specified by the manufacturer. Microarray results were extracted using Agilent Feature Extraction software Ver. 9.5.3.1 (Agilent Technologies) and analyzed using Gene Spring 12.0 software (Agilent Technologies) to obtain gene expression changes.

Follow-up

After curative surgery, all patients were followed every 3-6 months for 2 year, then every year or until death. The median follow-up for the entire cohort was 37.0 months (range: 3-71). The follow-up of all patients who were included in this study was completed in September 2012. B ultrasonography, CT scans, chest X-ray, and endoscopy were obtained with every visit.

Statistical analysis

Differences in the different variables of GC patients were estimated using the x^2 test for categorical data and independent-paired Student's t test for continuous variables. The qualitative correlation analysis was used the x^2 test, and linear correlation analysis was adopted as the quantitative detection method for the various clinicopathologic variables and biomarkers. All statistical calculations were performed using PASW Statistics 18 software. *P* value less than 0.05 was considered significant.

Results

Patient demographics and survival analyses

The clinicopathologic characteristics of 107 GC patients were shown in **Table 1**. The median OS of all patients was 21 months, and 20 (18.7%) patients were alive when the follow-up was over. The age range of all patients was between 23 and 79. The mean number of dissected lymph nodes was 24.7 ± 9.7, and that of metastatic lymph nodes was 6.81 ± 4.5. With the univariate analysis, five factors were identified to have statistical associations with the OS of gastric cancer patients after curative surgery. They were as follows: N stage, extent of metastatic lymph nodes, gender, STAT3 expression in GC tissue, pSTAT3 expression in GC tissue, VEGF-C expression in GC tissue, and VEGF-D expression in GC tissue. All above six factors were included in a multivariate Cox proportional hazards model (forward stepwise procedure) to adjust for the effects of covariates. In that model, only N stage and pSTAT3 expression in GC tissue were identified to be independent factors of the OS of GC patients (**Table 2** and **Figure 1**).

STAT3, pSTAT3, VEGF-C and VEGF-D expression in the GC and adjacent non-tumor tissues

Immunohistochemical staining and semi-quantitative RT-PCR were adopted to assess the protein expression and mRNA expression levels of STAT3, VEGF-C, and VEGF-D in the 107 GC tissues and the matched adjacent non-tumor tissues which were retrieved from the GC samples after surgery. Besides, pSTAT3 protein expression was also assessed in the 107 GC tissues and the matched adjacent non-tumor tissues by using the immunohistochemical staining. STAT3, pSTAT3, VEGF-C, and VEGF-D protein expression in GC tissues were significantly higher than those in adjacent non-tumor tissues, respectively ($_{pSTAT3}$ = 0.035, p_{pSTAT3} = 0.014, P_{VEGF-C} = 0.009, P_{VEGF-D} = 0.031) (**Figure 2**). The relative mRNA expression value of STAT3, VEGF-C, and VEGF-D in GC tissues were significantly much higher than those in adjacent non-tumor tissues, respectively (3.15 ± 1.32 VS 1.13 ± 0.64, p_{STAT3} = 0.004; 2.26 ± 0.83 VS 0.40 ± 0.12, p_{VEGF-C} = 0.007; 1.26 ± 0.55 VS 0.75 ± 0.29, p_{VEGF-D} = 0.041) (**Figure 3A**).

In this study, we demonstrated that STAT3 mRNA expression was much higher in each GC cell line than that in GES-1 cell line (p_{AGS} = 0.014, p_{SUN} = 0.020, $p_{KATO-III}$ = 0.026, $p_{BGC-823}$ = 0.023, $p_{MGC-803}$ = 0.019, $p_{SGC-7901}$ = 0.018, p_{HGC-27} = 0.014). Similarly, VEGF-D mRNA expression

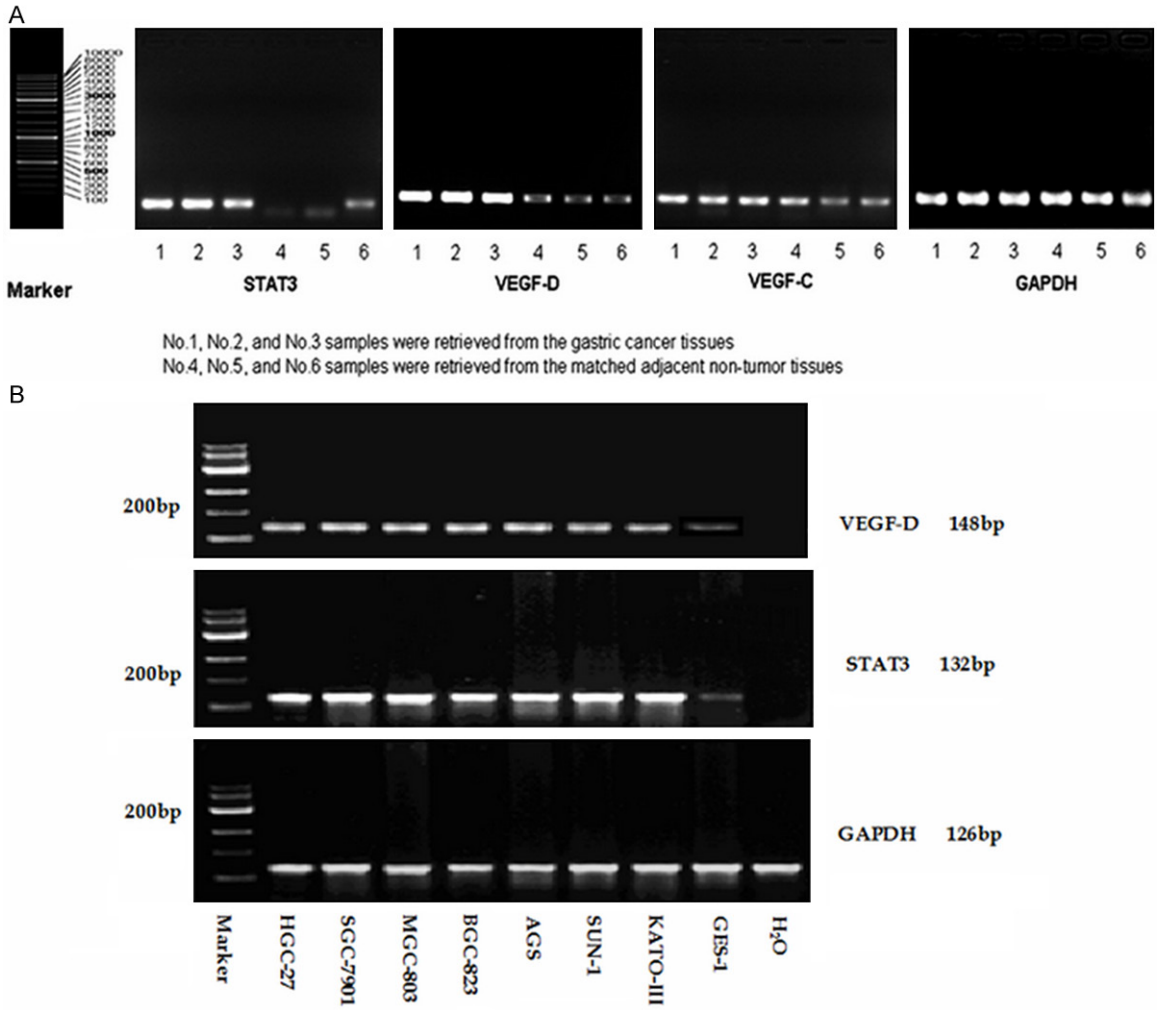

Figure 3. STAT3 and VEGF-D mRNA expression detection (semi-quantitative RT-PCR) in GC tissue and matched adjacent non-tumor tissue.

was also demonstrated to be significantly higher in each GC cell line than that in GES-1 cell line (p_{AGS} = 0.012, p_{SUN} = 0.025, $p_{KATO-III}$ = 0.017, $p_{BGC-823}$ = 0.015, $p_{MGC-803}$ = 0.009, $p_{SGC-7901}$ = 0.014, p_{HGC-27} = 0.007) (**Figure 3B**). However, VEGF-C mRNA expression failed to show much higher in each GC cell line than that in GES-1 cell line (p_{AGS} = 0.064, p_{SUN} = 0.435, $p_{KATO-III}$ = 0.108, $p_{BGC-823}$ = 0.058, $p_{MGC-803}$ = 0.235, $p_{SGC-7901}$ = 0.083, p_{HGC-27} = 0.072) (**Figure 3B**).

Correlation analysis between lymph node metastasis and STAT3, pSTAT3, VEGF-C and VEGF-D expression in the GC tissues

With the qualitative correlation analysis (x^2 test), we demonstrated that the sub-classification of the lymph node metastatic count (N stage) was significantly associated with the immunohistochemical expression (negative, or positive) of STAT3 (p = 0.021), pSTAT3 (p = 0.004), VEGF-C (p = 0.062), and VEGF-D (p = 0.034) in the 107 GC tissues, respectively. The STAT3 protein expression was significantly associated with VEGF-C protein expression (p = 0.106) and VEGF-D protein expression (p = 0.025) in the 107 GC tissues, respectively. The pSTAT3 protein expression was significantly associated with VEGF-D protein expression (p < 0.001) in the 107 GC tissues, respectively.

With the linear correlation analysis (quantitative detection method), we also demonstrated that the number of the lymph node metastasis was significantly associated with the relative expression values of STAT3 mRNA (p = 0.018), VEGF-C mRNA (p = 0.045), and VEGF-D mRNA (p = 0.037) in the 107 GC tissues, respectively. Furthermore, we found that STAT3 mRNA expression was significantly associated with

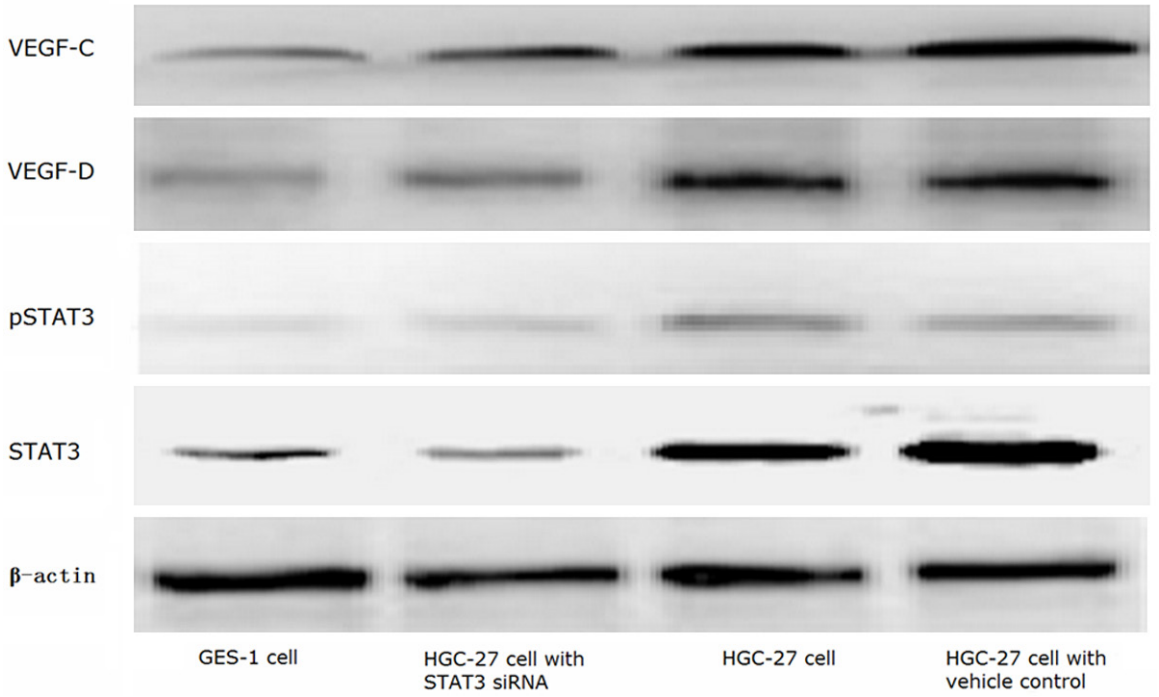

Figure 4. A. STAT3, pSTAT3, VEGF-C and VEGF-D mRNA expression (RT-PCR) in HGC-27, HGC-27 with STAT3 siRNA transfection, HGC-27 with vehicle control, and GES-1 cells; B. STAT3, pSTAT3, VEGF-C and VEGF-D protein expression (Western Blot) in HGC-27, HGC-27 with STAT3 siRNA transfection, HGC-27 with vehicle control, and GES-1 cells.

VEGF-D mRNA expression (p = 0.003) in the 107 GC tissues rather than VEGF-D mRNA expression (p = 0.115).

Inhibition of STAT3 expression leads to decrease of VEGF-D expression in GC cell

We adopted a siRNA approach to investigate the molecular regulation of STAT3 in the GC cell. To obtain the high-transfection efficiency and maintain low STAT3 protein level, we treated STAT3 positive HGC-27 cell line twice with either 3 nmol/L of specific siRNA against STAT3 or control siRNA during 2 days. On day 2, STAT3 and pSTAT3 protein expression level was measured by Western blot after the cells were harvested. We found a 60% to 70% decrease of STAT3 protein and a 70% to 80% decrease of pSTAT3 protein compared with the control in HGC-27 cell line. To elucidate if STAT3 down-regulation is effective for inhibition the expression of VEGF-C and VEGF-D in GC cell, we detected the protein expression of VEGF-C and VEGF-D in HGC-27 cell line with the western blot analysis. STAT3 down-regulation was identified to be sufficient to inhibit the protein expression of VEGF-C and VEGF-D in HGC-27 cell line. We found a 20% to 30% decrease of VEGF-C protein and a 70% to 80% decrease of VEGF-D protein compared with the control in HGC-27 cell line (**Figure 4**).

With the RNA microarray analyses, the significant expression decreases of 5295 genes were found in the HGC-27 cell with transfection of STAT3 siRNA. Of these genes, VEGF-C and VEGF-D were demonstrated to be down-regulated respectively. The relative RNA expression of VEGF-C decreased only 1.036-fold in the HGC-27 cell after STAT3 siRNA transfection. However, the relative RNA expression of VEGF-D significantly decreased 5.568-fold in the HGC-27 cell after STAT3 siRNA transfection.

Discussion

GC has an intensive propensity for lymphatic spread. STAT3 activation is identified to be involved in cell proliferation, apoptosis, differentiation, and other physiological activities. STAT3 constitutive activation was demonstrated to be significantly associated with lymph node metastasis in many different cancers [9, 12, 14-16]. Suppressors of cytokine signaling 3 (SOCS-3), an endogenous inhibitor of STAT3, was identified to be negatively related to the

lymph node metastasis from solid malignancies [17, 18]. In this study, we demonstrated that the immunohistochemical expression of STAT3 was significantly associated with lymph node metastasis from GC. Meanwhile, phosphorylated STAT3 expression and lymph node metastasis were validated to be the independent predictors of the OS of the GC patients by using the multivariate survival analysis. Therefore, we did intend to further analyze the biological mechanism between STAT3 and lymph node metastasis from GC. Theoretically, lymph node metastasis should be involved in the process of lymphangiogenesis from GC.

Recently, several authors have reported that STAT3 play a role in GC lymphatic metastases [11, 19]. As the most critical endogenous molecules, VEGF-C and VEGF-D were identified to be the key factors inducing the lymphangiogenesis to promote the lymph node metastasis, which was negatively associated with the prognosis of GC patients [20-24]. Our results showed that the high level expression of VEGF-C and VEGF-D was significantly related the abnormal activation of STAT3 in the GC tissues. In general, VEGF-C and VEGF-D have been defined as lymphangiogenic growth factors and play an important role in tumor lymphangiogenesis via activation of the VEGF receptor (VEGFR)-3, which is expressed in lymphatic endothelial cells [25, 26]. Chen et al [14] demonstrated that STAT3 protein expression was significantly related to the expression of VEGF-C and VEGF-D in breast cancer, which was considered that STAT3 could induce the abnormal level expression of the endogenous molecules to promote the lymphangiogenesis and lymphatic metastasis. To date, the downstream molecules of STAT3 are not clearly elucidated. In our previous study, we found that STAT3 protein potentially regulated the target molecules including the apoptosis, cell-survival, and angiogenesis [9]. Huang et al [11] demonstrated that STAT3 was an important central cytoplasmic transcription factor and regulated the tumor metastasis, which enhanced the lymphatic metastasis through VEGF-C. At present study, we demonstrated that VEGF-D expression was significantly reduced with transfection of STAT3 siRNA in HGC-27 GC cell. On the other hand, we found that VEGF-D and STAT3 protein and mRNA expression in GC tissue was obviously higher than that in the matched adjacent non-tumor tissues. Meanwhile, we also found that the enhanced VEGF-D protein and mRNA expression in GC was significantly related to STAT3 expression. In view of the results of mRNA microarray analyses, STAT3 was identified to be intensively potential regulator of VEGF-D in HGC-27 GC cell. Therefore, we deduced that the abnormal STAT3 activation might be a key molecular switch to enhance the VEGF-D expression in GC.

In a conclusion, our findings show that STAT3 can induce the lymphangiogenesis by enhancing the VEGF-D expression rather than VEGF-C expression, which can promote the lymphatic metastasis including the lymph node metastasis from GC. STAT3/VEGF-D signaling pathway might be considered as a new molecular target for inhibition the nodal metastasis in GC.

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Disclosure of conflict of interest

None.

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