

Soluble interleukin-2 receptor as a factor associated with angiogenesis in gastric cancer

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Abstract. Angiogenesis serves a role in the growth, metastasis and prognosis of tumors. The aim of the present study was to evaluate the angiogenic ability and clinical significance of the immune biomarker soluble interleukin-2 receptor (sIL-2R) in gastric cancer (GC) patients. Serum levels of sIL-2R were measured in 35 GC patients with different stages of disease and 32 healthy individuals, and it was investigated whether the levels were associated with angiogenesis factors, including vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)- β 1. Human umbilical vein endothelial cells (HUVECs) were pretreated with or without recombinant human (rh)sIL-2R, VEGF and TGF- β 1 for 24 h, and then the HUVECs were harvested to determine the degree of angiogenesis. The supernatants were also collected for VEGF and TGF- β 1 testing. Serum levels of sIL-2R were higher in GC patients than in healthy individuals, as were the levels of VEGF and TGF- β 1. In addition, serum levels of sIL-2R were positively associated with the levels of VEGF and TGF- β 1. Angiogenesis of HUVECs was also increased by rhIL-2R pretreatment. VEGF and TGF- β 1 secretion were also increased in supernatants that were pretreated with rhIL-2R. The results of the present study suggested that serum levels of sIL-2R contributes to the pathophysiology of GC progression and may be used as a prognostic biomarker for GC.

Introduction

Gastric cancer (GC) is a heterogeneous disease that evolves from various genetic and epigenetic alterations (1). According

to global cancer statistics (2012), GC is the 5th most common cancer and is the 3rd most common cause of cancer-associated death. In 2012, an estimated 951,600 new stomach cancer cases and 723,100 mortalities occurred worldwide, and the incidence rates were highest in Eastern Asia (particularly in Korea, Mongolia, Japan and China) (2). The majority of patients are treated with surgical resection and chemotherapy at the time of diagnosis; however, the overall 5-year survival rate of GC patients remains unsatisfactory (3-5). Therefore, investigating the underlying mechanisms of effector molecules and signaling pathways that promote the initiation and progression of GC require further investigation.

Previous studies reported that angiogenesis serves an important role in the pathogenesis of certain cancers, including GC (6,7). Vascular endothelial growth factor (VEGF) is an angiogenic cytokine that specifically binds to receptor tyrosine kinases (RTKs), including VEGF receptor (R)1 (encoded by the *FLT1* gene), VEGFR2 (encoded by the *KDR* gene) and VEGFR3 (encoded by the *FLT4* gene) (8,9). Reduced paracrine secretion of VEGF from tumor cells may suppress angiogenic activity (10). An additional angiogenic cytokine, transforming growth factor- β 1 (TGF- β 1) is an important transcriptional regulator of the extracellular matrix, and high expression of TGF- β 1 is associated with significantly poor overall disease-free survival (11). This suggests that angiogenesis is tightly controlled by angiogenic cytokines and inflammatory factors in GC.

Serum soluble interleukin-2 receptor (sIL-2R) is an important monitoring index which reflects cellular immunity function in the body (12). It has been reported that sIL-2R levels may be used as an independent prognostic index in follicular lymphoma patients (13). High sIL-2R levels in patients with tumors may inhibit the proliferation of T cells (14). However, whether sIL-2R is involved in progression of GC remains to be determined. The aim of the present study was to detect the serum levels of sIL-2R in patients with GC and to determine whether they are associated with VEGF and TGF- β 1, which may provide an insight into whether sIL-2R may be used as a clinical biomarker for GC.

Materials and methods

Patients. The original research was approved by the Medical Ethics Committee of Henan Provincial People's Hospital,

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Table I. Clinical characteristics of gastric cancer patients and healthy controls.

Parameter	Healthy controls	Gastric cancer patients
Total	32	35
Age (years)	57±7.54	62±17.21
Sex (male/female)	17/15	19/16
Tumor location in the stomach		
Upper	-	11
Middle	-	15
Lower	-	9
Stage		
II A-B	-	2
III A-C	-	24
IV	-	9

Data are presented as the mean ± standard deviation.

People's Hospital of Zhengzhou University (approval no. KY2012-005; Henan, China) and written informed consent was provided by the patients. A total of 35 newly diagnosed GC patients were enrolled between January 2013 and April 2015. Individuals with hypertension and diabetes and those who had received surgery were excluded. A total of 32 age- and sex-matched healthy individuals also enrolled at Henan Provincial People's Hospital were used as controls. The characteristics of subjects enrolled in the study are presented in Table I.

Serum cytokine measurements. All blood samples were collected, mixed with EDTA and centrifuged at 1,000 x g for 15 min at room temperature, and the supernatants (serum) were stored at -80°C until analysis. Soluble IL-2R (CSB-E04629H, CUSABIO; Flarebio Biotech LLC, Wuhan, China), VEGF and TGF-β1 in the serum samples were determined using ELISA kits (VEGF, DVE00; TGF-β1, DB100B; R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. The final concentration in each sample was calculated by interpolation of the standard curve.

Angiogenic capacity. The angiogenic capacity of vascular-like structures was determined using human umbilical vein endothelial cells (HUVECs) that were purchased from Cell Resource Center, China infrastructure of cell line resources (Beijing, China). HUVECs (2x10⁵/well) were pretreated with recombinant human (rh)sIL-2R (200 ng/ml; PromoCell GmbH, Heidelberg, Germany), VEGF (20 ng/ml; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), TGF-β1 (10 ng/ml; ProSpec-Tany TechnoGene, Ltd., East Brunswick, NJ, USA) or culture medium respectively for at 37°C and 5% CO₂ for 24 h, then cells were collected and seeded into 96-well plates coated with 50 μl Matrigel® (15) (BD Biosciences, Franklin Lakes, NJ, USA). The plates were maintained at 37°C and 5% CO₂ for 6 h. Tubes were defined as straight cellular extensions

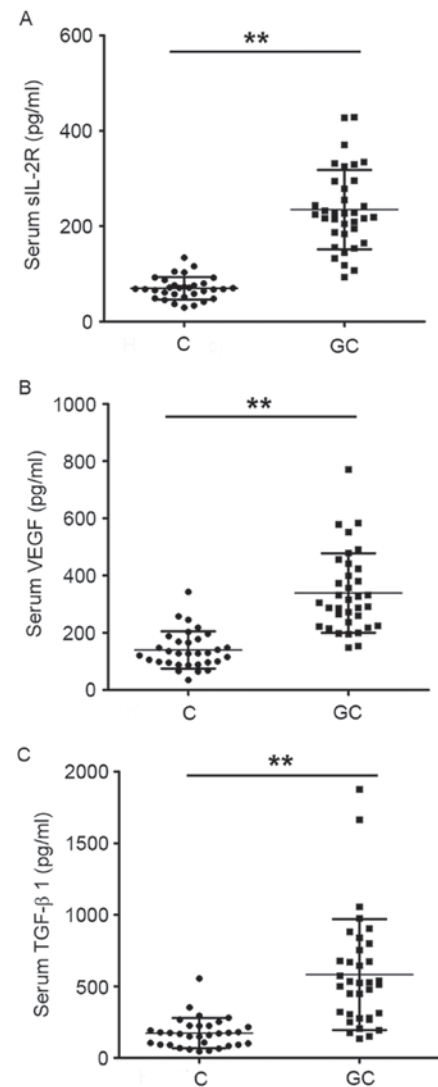


Figure 1. Serum levels of (A) sIL-2R, (B) VEGF and (C) TGF-β1 in GC patients and healthy controls. **P<0.01 vs. C. sIL-2R, soluble interleukin-2 receptor; VEGF, vascular endothelial growth factor; TGF-β1, transforming growth factor-β1; GC, gastric cancer; C, control.

forming a closed loop and digital images (at x200 magnification) of each well were taken by an inverted microscope (Leica QUIPS; Leica Microsystems, Ltd., Milton Keynes, UK) and three random digital images in each well were counted.

Statistical analysis. Results are expressed as the mean ± standard deviation. Student's t-test was used to determine differences in serum cytokines between GC patients and healthy individuals. Associations between parameters were calculated by the Spearman's rank or Pearson correlation coefficient. P<0.05 was considered to indicate a statistically significant difference.

Results

Serum levels of sIL-2R, VEGF and TGF-β1 were significantly increased in GC patients compared with healthy individuals (P<0.01; Fig. 1A-C). It was also revealed that there were significant positive associations between serum levels of sIL-2R and VEGF (R²=0.1369, P=0.0287; Fig. 2A)

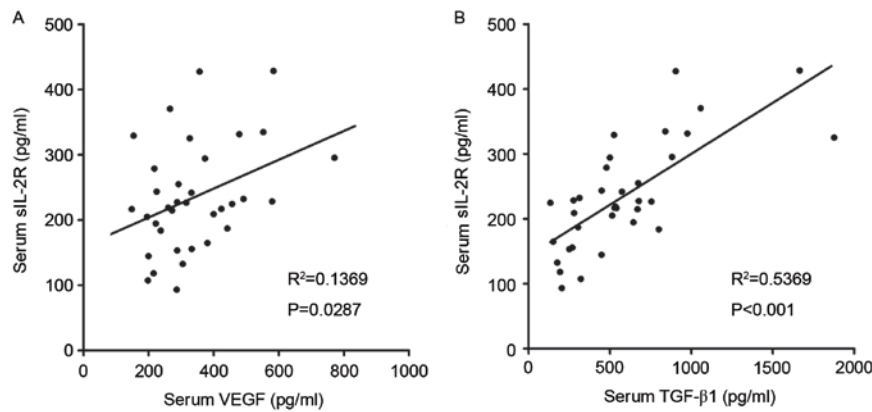


Figure 2. Correlational analysis of sIL-2R with VEGF and TGF- β 1. Serum levels of sIL-2R positively correlate with (A) VEGF and (B) TGF- β 1 in GC patients. sIL-2R, soluble interleukin-2 receptor; VEGF, vascular endothelial growth factor; TGF- β 1, transforming growth factor- β 1; GC, gastric cancer.

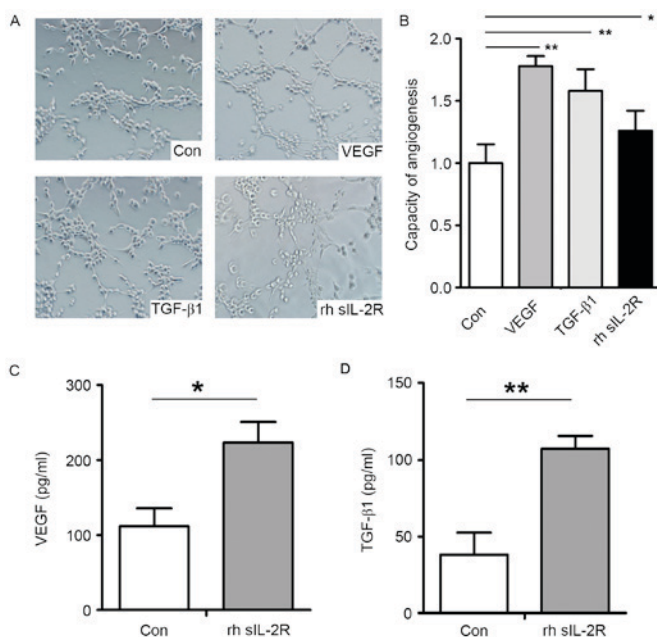


Figure 3. (A) Representative images (magnification, $\times 200$) and (B) quantitative analysis of tube formation in HUVECs pretreated with or without rh sIL-2R, VEGF or TGF- β 1. Results are expressed relative to the control. (C) VEGF and (D) TGF- β 1 secretion in the supernatant of the control or HUVECs treated with rh sIL-2R. Data are expressed as the mean \pm standard deviation and experiments were performed in triplicate. * $P < 0.05$ and ** $P < 0.01$ vs. Con. rh sIL-2R, recombinant human soluble interleukin-2 receptor; VEGF, vascular endothelial growth factor; TGF- β 1, transforming growth factor- β 1; Con, control.

and TGF- β 1 ($R^2=0.5369$, $P < 0.001$; Fig. 2B). The angiogenic capacity of HUVECs was enhanced after pretreatment with rh sIL-2R ($P < 0.05$), VEGF ($P < 0.01$) and TGF- β 1 ($P < 0.01$) compared with the control group (Fig. 3A and B). In addition, rh sIL-2R pretreatment significantly increased the secretion of VEGF ($P < 0.05$; Fig. 3C) and TGF- β 1 ($P < 0.01$; Fig. 3D) in HUVECs.

Discussion

It is now accepted that angiogenesis is a crucial step involved in tumor pathogenesis, and angiogenic and anti-angiogenic signaling pathways provide a switch for tumor progression (16,17).

Investigating the specific contribution of pro-angiogenic factors in tumors may lead to the identification of therapeutic targets and reliable biomarkers in the clinic. In the present study, serum sIL-2R, VEGF and TGF- β 1 levels were measured in GC patients and healthy controls. It was revealed that serum levels of sIL-2R, VEGF and TGF- β 1 were significantly increased in GC patients compared with healthy individuals. In addition, sIL-2R was significantly associated with VEGF and TGF- β 1, which are important angiogenetic factors. This data suggested that sIL-2R may be an angiogenesis marker in GC.

sIL-2R is a glycoprotein which is derived from the α chain of IL-2 receptors of the mononuclear and T-cell membranes, and its molecular weight is 45 kDa (18). The secretion of sIL-2R serves a significant role in regulating cell immune function. When IL-2 binds to sIL-2R, differentiation of memory T cells into effector T cells is promoted to help fight infection. Previous studies demonstrated that the expression levels of sIL-2R were increased in the serum of patients with metastatic melanoma, and appeared to aid the prediction of patient outcome (19). Increased serum sIL-2R expression levels have an effect on a hamster cheek pouch carcinoma model subsequent to heavy-ion beam irradiation (20). This suggests that there may be an essential role for sIL-2R in the tumor microenvironment. In the present study, there were significantly increased serum sIL-2R levels in the serum of GC patients than healthy controls. This suggests that the secretion of sIL-2R serves an important role in GC progression.

TGF- β 1 is a prototypical member of the TGF protein superfamily, is involved in cell growth, differentiation, motility and angiogenesis, and is associated with a negative prognosis (21-23). In addition, TGF- β 1 induces VEGF expression in vascular endothelial cells (24), suggesting that TGF- β 1 is an important component of angiogenic activity. In previous studies, VEGF has been reported to be expressed in response to immunity and inflammation and also exerts a systemic influence on immune cell development and function in tumors (25,26). Incremental levels of circulating VEGF inhibit T cell immune responses in colorectal cancer. In the present study, there was a significant positive association between serum levels of sIL-2R and VEGF and TGF- β 1. Therefore, the association between sIL-2R and angiogenesis was investigated *in vitro*. Pretreatment with rh sIL-2R significantly increased the

secretion of VEGF and TGF- β 1, and the angiogenic capacity of HUVECs was enhanced after rhIL-2R pretreatment. These results suggested that sIL-2R may serve a role in angiogenesis during GC progression. However, further study is required to establish if sIL2R is a factor that promotes the angiogenic capacity of HUVECs directly. The underlying mechanism of how sIL2R induces VEGF expression in the tumor microenvironment requires further investigation.

In conclusion, the results of the present study suggested that GC patients exhibit enhanced serum levels of sIL-2R, VEGF and TGF- β 1. sIL-2R was positively associated with VEGF and TGF- β 1. The angiogenic capacity of HUVECs was enhanced by pretreatment with sIL-2R. These results suggested that serum levels of sIL-2R serve a role in GC progression, and may be a marker of angiogenesis in GC.

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