RESEARCH ARTICLE

Plasma concentrations of Ang-1, Ang-2 and Tie-2 in gastric cancer

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ABSTRACT. ***Background/Aim:*** **Ang-1 and Ang-2 have both been identified as ligands for Tie-2, a receptor expressed on endothelial cells (EC). They play critical roles in angiogenesis, in concert with VEGF. Ang-1-binding to Tie-2 maintains and stabilizes mature vessels by promoting interactions between EC and the surrounding extra-cellular matrix. However, Ang-2 shows context-dependent, proangiogenic and antiangiogenic activities. Despite the rapidly accumulating histopathological data reporting differences in the expression of members of the Ang family on the surface of various normal and tumour cells, data for these growth factors in plasma from cancer patients, including gastric cancer, remain scarce. The aims of the present study were to measure the plasma concentrations of Ang-1, Ang-2 and Tie-2 in gastric cancer patients, and to assess the correlation between the concentrations of these factors and the stage of the tumor.** ***Patients and Methods:*** **The study cohort consisted of 50 patients (33 male, 17 female) with gastric cancer, ranging in age from 38 to 74 years (mean age 55.3±12.4), and 50 sex- and age-matched, healthy controls. Determinations of Ang-1, Ang-2 and Tie-2 were performed using the enzyme-linked immunosorbent assay (ELISA) method.** ***Results:*** **Concentrations of Ang-2 and Tie-2 were significantly higher in patients with gastric cancer than controls, while concentrations of Ang-1 were not statistically different between the groups. Concentrations of Ang-1, Ang-2 and Tie-2 were not statistically significantly different in gastric cancer patients with different stages of disease.** ***Conclusion:*** **Ang-2 and Tie-2 plasma concentrations might be useful, additional tumor markers in gastric cancer.**

Keywords: Angs, stomach neoplasms

Gastric cancer is rampant in many countries around the world. By some estimates, it is the fourth most common cancer worldwide [1]. The overall negative outcome for this neoplasm in western countries has not significantly improved over the last decades. So identification of prognostic and predictive factors that reflect the biology of gastric cancer (tumor spread and metastasis) is important for refining our assessment of prognosis and the selection of patients who may benefit from adjuvant systemic therapy [2].

Angiogenesis, the formation of new blood vessels from preexisting blood vessels, is a fundamental process in tumor growth and metastasis. Angiogenesis is regulated by several peptide and nonpeptide molecules. Among the most widely studied molecules are vascular endothelial growth factor (VEGF) and its receptor Flt-1, and the Ang family of molecules, Ang-1 (Ang-1) and Ang-2 (Ang-2), and their receptor Tie-2 [3-7].

Of the four angiopoietins identified (Ang-1 to Ang-4), the best characterized are Ang-1 and Ang-2. Ang-1 and Ang-2 have both been identified as ligands for Tie-2, a receptor expressed on endothelial cells (EC), and they play critical roles in angiogenesis, in concert with VEGF [3-8]. Ang-1 binding to Tie-2 maintains and stabilizes mature vessels by promoting interactions between EC and the surrounding extra-cellular matrix. However, Ang-2 shows context-dependent, proangiogenic and antiangiogenic activities. Ang-2 was first identified as a natural antagonist for Tie-2 that disrupts *in vivo* angiogenesis. Ang-2 is only up-regulated at sites of active vascular remodeling, which involves vessel destabilization and regression [6]. These destabilized vessels may undergo regression in the absence of VEGF; however, when VEGF is present, these destabilized vessels may undergo angiogenic changes.

Despite the rapidly accumulating, histopathological data reporting differences in the expression of members of the Ang family on the surface of various normal and tumour cells, data concerning these growth factors in plasma from gastric cancer patients are lacking.

So the aims of the present study were to measure the plasma concentrations of Ang-1, Ang-2 and Tie-2 in gastric cancer

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patients, to assess the correlation between the concentrations of these factors and the stage of the tumor, and to determine whether they might be considered as additional markers in patients with gastric cancer.

PATIENTS AND METHODS

Subjects

The study cohort consisted of 50 patients (33 male, 17 female) with gastric cancer ranging in age from 38 to 74 years (mean age 55.3±12.4), and 50 sex- and age-matched healthy controls free from inflammatory, neoplastic, atherosclerotic or connective tissue disease, who were recruited from hospital staff and attendees at hospital for check-ups. Gastric cancer patients were staged according to the 7th ed. American Joint Committee on Cancer (AJCC) TNM Staging Classification for Carcinoma of the Stomach [9]. According to AJCC staging, nine patients had stage I, 12 patients had stage II, 15 patients had stage III and 14 patients had stage IV disease. All patients had adenocarcinoma.

Ang-1, Ang-2 and Tie-2 concentrations were evaluated upon confirmation of cancer, and before the commencement of any type of treatment.

The subjects were patients of the Karaelmas University Hospital, Department of Medical Oncology, in Zonguldak, Turkey. Informed consent was obtained from every patient and control before enrollment into the study. The study was approved by the Ethical Committee for Scientific Studies at the Karaelmas University, Zonguldak, Turkey.

Measurement of cytokines

Blood samples were taken in the morning between 7:00 and 8:00 a.m., after an overnight fast. Blood was processed within one hour of collection, and plasma was aliquoted and stored at -80°C until analysis. Determinations of Ang-1, Ang-2 and Tie-2 were performed using commercial, enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems (Quantikine, R&D Systems Inc., 614 Mc Kinley Place NE, Minneapois 55413, USA; catalog numbers respectively, DANG10, DANG20, and DTE200), in accordance with the manufacturer's instructions. Plasma samples for Ang-1, Ang-2 and Tie-2 determinations were diluted with assay buffer 15-, 5- and 10- fold, respectively. All measurements were performed in duplicate and averaged.

Statistical analysis

All statistical analyses were conducted by using the SPSS 18.0 Statistical Software Prrogram (SPSS, Chicago, IL, USA). Statistical analyses were done using the nonparametric Mann-Whitney test, one-way analysis of variance, and Pearson's linear correlation. The results were considered statistically significant at $p<0.05$.

RESULTS

Table 1 shows the comparison of Ang-1, Ang-2, and Tie-2 concentrations between the groups. Concentrations of Ang-2 and Tie-2 were significantly higher in patients with gastric cancer than controls, while concentrations of Ang-1 were not statistically different between the groups.

Table 2 shows the comparison of Ang-1, Ang-2, and Tie-2 concentrations between the stages of disease in patients with gastric cancer. Concentrations of Ang-1, Ang-2 and Tie-2 were not statistically significantly different between the stages.

In this study an estimation of the correlation between factors was carried out. Correlations between Ang-1, Ang-2, and Tie-2 were as follows; Ang-1 *versus* Ang-2: r=-0.06 (p=0.04); Ang-1 *versus* Tie-2: r=0.12 (p=0.02) and Ang-2 *versus* Tie-2: r=0.14 (p=0.03). The correlations that were statistically significantly positive in the subgroup with stage III disease were Ang-1 *versus* Ang-2: r=0.34 (p=0.03) and Ang-2 *versus* Tie-2: r=0.48 (p=0.002), and in the subgroup with stage IV disease were Ang-1 *versus* Ang-2: r=0.38 (p=0.009) and Ang-2 *versus* Tie-2: r=0.67 (p=0.001) (*table 3*).

DISCUSSION

After analysis of angiopoietins expression in tumors, the question arises as to whether changes in angiopoietins concentrations in peripheral blood occur. Increased plasma concentrations of Ang-1 and Ang-2 were seen in breast, prostate and cervical cancer [10, 11], and increased plasma concentrations of Ang-2 in lung cancer [12]. An increased concentration of Tie-2 was detected in colorectal cancer [13] and also in relation to metastasis [14]. In our study, plasma concentrations of Ang-2 and Tie-2 were significantly higher in gastric cancer patients than in controls, while there was no difference in Ang-1 concentrations between these groups. This is the first study in the literature measuring plasma concentrations of Ang-1, Ang-2 and Tie-2 in gastric cancer.

Changes in angiopoietins concentrations and their receptor expression have been frequently observed in cancer. Results of investigations related to Ang-2 expression in various tumors are unequivocal. Expression was usually increased [15-18]. However, on the subject of Ang-1, opinions are divided. For example, the overexpression of Ang-1 has been observed in colorectal adenocarcinoma and breast cancer [15, 19]. Tie-2 was also overexpressed in tumors [15]. There is relatively little information regarding the correlation between angiopoietins expression and

Table 1
Plasma concentrations of Ang-1, Ang-2, and Tie-2 in gastric cancer patients and control group (median, min-max).

	Gastric cancer	Controls	P value
Ang-1 (pg/mL)	8406 (1254-40830)	6013.5 (937.5-35496)	0.120
Ang-2 (pg/mL)	3684.5 (910.5-15000)	2025.0 (745.5-5202.5)	0.001
Tie-2 (ng/mL)	22 (9-36)	17 (2-26)	0.045

Table 2
Plasma concentrations of Ang-1, Ang-2, and Tie-2 in gastric cancer patients with different stages of disease (median, min-max) (p>0.05).

	Stage I	Stage II	Stage III	Stage IV
Ang-1 (pg/mL)	8196 (1,254-3,8461.5)	8463 (2,193-40,833)	8494.5 (2,157-39,631.5)	8,470.5 (1,509-40,509)
Ang-2 (pg/mL)	3650 (910.5-13,500)	3713 (1,239-15,000)	3672.5 (1,503-14,480)	3702.5 (1,118-13,900)
Tie-2 (ng/mL)	21 (12-36)	23(9-34)	22 (10-35)	21 (11-33)

Table 3
Correlation (r) between estimated factors in subgroups with stage III and IV disease.

		Ang-2		Tie2	
		III	IV	III	IV
Ang-1	III	r=0.34 (p=0.03)		r=-0.06 (p=0.09)	
	IV		r=0.38 (p=0.009)		r=-0.16 (p=0.07)
Ang-2	III			r=0.48 (p=0.002)	
	IV				r=0.67 (p=0.001)

clinical features or prognosis, although some clinical studies on angiopoietins expression have been conducted in a variety of tumors, including gastric cancer.

The potential correlation between levels of angiopoietins within the serum and the tumor itself is not also well understood. We could find only one study in the literature concerning this correlation [20]. In the study, Figueroa-Vega *et al*. prospectively examined serum levels of Tie-2, Ang-1, and Ang-2 using ELISA, in 42 patients with proven gastroenteropancreatic neuroendocrine tumors (GEP-NETs), and 27 controls. They also determined the expression of the Ang/Tie-2 system in freshly isolated, peripheral blood monocytes and in tumor cells from malignant primary tumors and/or liver metastases samples from GEP-NET patients using flow cytometry and/or RT-PCR. Furthermore, the function of the Ang/Tie-2 system in monocytes from controls and patients was assessed using a chemotaxis assay. GEP-NET patients showed enhanced serum levels of the soluble form of Tie-2 (sTie-2), Ang-1, and Ang-2 (P<0.05 in all cases), compared to controls. sTie-2 and Ang-2 levels were significantly higher in GEP-NETs with metastases compared to those with no metastases. In addition, a significant correlation was detected between Ang-2 levels and:

– chromogranin A (r=0.71),

– sTie-2 concentrations (r=0.60),

– 5-hydroxy-indole acetic acid excretion (r=0.81, P<0.01 in all cases).

Furthermore, using immunohistochemistry and RT-PCR, an enhanced expression of Ang-1, Ang-2, and Tie-2 in freshly isolated tumor cells from GEP-NET was observed. Interestingly, an enhanced expression and function of Tie-2 was detected in monocytes from GEP-NET patients.

Moon *et al*. examined expression of Ang-1, Ang-2, and Tie-2 mRNAs and proteins in gastric cancers using *in situ* hybridization and immunohistochemistry. They also investigated the relationship between their expression and the differentiation of cancer cells, lymph node metastasis, tumor size, depth of cancer cell invasion, TNM staging and microvessel density (MVD). The expression of Ang-1, Ang-2, and Tie-2 mRNA in cancer cells correlated significantly with the MVD (p<0.001, <0.001 and =0.019, respectively). Ang-1 and Tie-2 correlated positively with advanced gastric cancers (p<0.05), larger cancers having higher positive rates of Ang-1, Ang-2, and Tie-2 mRNA expression (p<0.001, =0.010 and =0.039, respectively). Significantly positive correlations were also found between mRNA expression of Tie-2 and those of Ang-1 and Ang-2 (p<0.01 and 0.001, respectively) [21]. Wang *et al*. compared the expression of Ang-1, Ang-2 and Tie-2 using immunohistochemistry in 53 gastric cancer and 23 normal gastric mucosa samples. Results revealed that Ang-2 expression was increased significantly in gastric cancer tissues (74%), and correlated with a higher TNM stage, lymph node metastases, as well as distant metastases. The expression of Ang-1 was also increased in cancerous tissues (66%) and was associated significantly with the degree of differentiation. In addition, expression of Ang-2 and its receptor Tie2, was shown to be higher in 12 pairs of gastric cancer tissue samples than in corresponding adjacent samples using Western blot, while Ang-1 expression showed great heterogeneity. Furthermore, the expressions of Ang-1 and Ang-2 were almost positive in eight gastric cancer cell lines [22]. Nakayama *et al*. studied expression of Tie-1 and two receptors, and Ang-1, -2 and -4 in eighty-nine cases of surgically-resected human gastric adenocarcinoma. Of these, 60 (67.4%), 61 (68.5%), 69 (77.5%), 75 (84.3%), and 47 cases (52.8%) showed positive staining in the cytoplasm of the carcinoma cells for the Tie-1 and 2 and Ang-1, 2 and 4 proteins, respectively. The expression of Ties and angiopoietins correlated significantly with several types of histological differentiation and several clinicopathological factors [23]. Recently, Jo *et al*. assessed the relationship between preoperative serum Ang-2 and lymph node metastasis in patients with early gastric cancer. The serum levels of Ang-2 were quantified using immunoassay. Intra- and peritumor lymphatic vessel density (I-LVD and P-LVD) were measured after immunohistochemical staining. The relationship between serum Ang-2 levels and other prognostic variables (tumor size, histological type, depth of tumor invasion, I-LDV, P-LDV, presence of lymph node involvement, and distant metastases) were then subjected to univariate and multivariate linear regression analyses.

They found that increased serum Ang-2 levels were associated with positive lymph node involvement and this finding was significant on univariate (P=0.008) and multivariate logistic regression analysis (P=0.011) [24].

Ang-1, Ang-2 and Tie-2 concentrations were not statistically significantly different in gastric cancer patients with different stages of disease. The correlation between the factors studied in the whole group was weak, while in the subgroups with different stages of disease, correlations were stronger. These results support earlier thoughts that at each clinical stage of tumor, angiogenesis may be in different phases [11, 25, 26].

In conclusion, Ang-2 and Tie-2 plasma concentrations may be useful, additional tumor markers in gastric cancer, however, these results need to be confirmed in prospective studies with larger cohorts of patients.

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