RESEARCH ARTICLE

Plasma concentrations of angiopoietin-1, angiopoietin-2 and Tie-2 in colon cancer

Hüseyin Engin (MD)[1], Yücel Üstündağ (MD)[2], İshak Özel Tekin (MD)[3], Ayla Gökmen (MD)[4], Şehmuz Ertop[4], Sevil Uygun İlikhan[5]

[1] Karaelmas University, Department of Internal Medicine, Division of Medical Oncology, Zonguldak-Turkey
[2] Karaelmas University, Department of Internal Medicine, Division of Gastroenterology, Zonguldak-Turkey
[3] Karaelmas University, Department of Immunology, Zonguldak-Turkey
[4] Karaelmas University, Department of Internal Medicine, Division of Hematology, Zonguldak-Turkey
[5] Karaelmas University, Department of Internal Medicine, Zonguldak-Turkey

Correspondance: M.D. Hüseyin Engin, Karaelmas University Faculty of Medicine, Department of Internal Medicine, Division of Medical Oncology, 67600 Zonguldak-Turkey
<bulengin@yahoo.com>

ABSTRACT. *Background/Aim:* **despite the rapidly accumulating histopathological data reporting differences in the expression of members of the angiopoietin family on the surface of various normal and tumour cells, data for these growth factors in plasma from cancer patients, including colon cancer, are scarce. The aims of the present study were to measure the plasma concentrations of Ang-1, Ang-2 and Tie-2 in colon 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 included 36 patients (18 male, 18 female) with colon cancer (mean age 52.6 ± 15.0), and 36 sex- and age-matched, healthy controls who were free of inflammatory, neoplastic, atherosclerotic and connective tissue disease, recruited from hospital staff and attendees at hospital for check-up. Concentrations of Ang-1, Ang-2 and Tie-2 were measured using the enzyme-linked immunosorbent assay (ELISA) method.** *Results:* **concentrations of Ang-2 (median 3,188.0 pg/mL, min: 1,070.5-max: 5,765.5) and Tie-2 (median 22 ng/mL, min:12-max:46) were significantly higher in patients with colon cancer, while concentrations of Ang-1 were not statistically different between the groups. Furthermore, concentrations of Ang-2 (median 4,292.0 pg/mL, min: 3,090.0-max: 5,765.5) were found to be significantly higher in stage III patients compared to stage II patients, whereas no difference was found between the concentrations of Ang-1 and Tie-2 in different colon cancer stages.** *Conclusion:* **plasma concentrations of Ang-1, Ang-2 and Tie-2 may be valuable, additional, tumor markers in colon cancer that should be tested in further trials.**

Key words: Angs, colorectal neoplasms

Colon cancer is one of the most common malignancies worldwide. It has been shown that angiogenesis is an independent prognostic factor for this type of cancer [1]. Tumor angiogenesis, the formation of new blood vessels from the existing vasculature, is a complex, dynamic process consisting of extra-cellular matrix remodelling, endothelial cell proliferation and capillary differentiation, coordinated by several classes of growth factors acting through cognate tyrosine kinase receptors. It is an essential process in the development and progression of malignancy. Angiogenesis is regulated by several peptides and nonpeptide molecules. Among the most widely studied molecules are vascular endothelial growth factor (VEGF) and its receptor Flt-1, and the angiopoietin family of molecules, angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2), and their receptor Tie-2 [2-6].

Of the four identified angiopoietins (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 [2-7]. Ang-1 was first identified as a major activator of Tie-2, resulting in a downstream activation of the phosphatidylinositol 3-kinase/Akt survival pathway, thereby promoting EC survival. Ang-1 binding to Tie-2 maintains and stabilizes mature vessels by promoting interactions between EC and 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. Studies of Ang-2 knockout animals showed that Ang-2 is not required for embryonic vascular development, but is needed for postnatal, angiogenic remodeling [8]. Ang-2 is only up-regulated at sites of active vascular remodeling, which involves vessel destabilization and regression [5]. These destabilized vessels may undergo regression in the absence of VEGF; however, when VEGF is present, these destabilized vessels may undergo angiogenic changes.

The Ang-1 and Ang-2/Tie-2 family have been reported to be involved in the pathogenesis of several kinds of

cancers, including astrocytoma, breast cancer, prostate cancer, colorectal cancer, gastric cancer, thyroid cancer, cervical cancer [9-15].

Despite the rapidly accumulating histopathological data reporting differences in the expression of members of the angiopoietin family on the surface of various normal and tumour cells, data for these growth factors in plasma from cancer patients are scarce. Thus far, there is no published study in the English literature concerning the concentrations of these growth factors in the plasma of colon cancer patients.

The aims of the present study were to measure the plasma concentrations of Ang-1, Ang-2 and Tie-2 in colon cancer patients, and to assess the correlation between the concentrations of these factors and the stage of the tumor.

PATIENTS AND METHODS

Subjects

The study cohort consisted of 36 patients (18 male, 18 female) with colon cancer, ranging in age from 34 to 79 years (mean age 52.6 ± 15.0) and 36 sex- and age-matched, healthy controls free of inflammatory, neoplastic, atherosclerotic or connective tissue disease who were recruited from hospital staff and attendees at hospital for check-up. Colon cancer patients were staged according to the 7th ed. American Joint Committee on Cancer (AJCC) TNM Staging Classification [16]. Nineteen patients were classified as stage II, and 17 patients as stage III. All patients had adenocarcinoma.

Ang-1, Ang-2 and Tie-2 concentrations were evaluated upon ascertainment of cancer, before commencing 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 Karaelmas University, Zonguldak, Turkey. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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 serum was aliquotted 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 McKinley Place NE, Minneapolis 55413, USA; catalog numbers respectively, DANG10, DANG20, and DTE200), following 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 analysis was performed using the nonparametric Mann-Whitney test 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 colon cancer than controls, while there were no differences in the concentrations of Ang-1.

Table 2 shows the results of comparison of Ang-1, Ang-2, and Tie-2 concentrations between stage II and stage III colon cancer patients. Concentrations of Ang-2 were significantly higher in stage III patients, whereas concentrations of Ang-1 and Tie-2 were no different between the stages.

In this study, an estimation of factors correlation was carried out. Correlations between Ang-1, Ang-2, and Tie-2 were as follows; Ang-1 versus Ang-2: $r = -0.08$ ($p = 0.08$); Ang-1 versus Tie-2: $r = 0.16$ ($p = 0.04$) and Ang-2 versus Tie-2: $r = 0.18$ ($p = 0.01$). The correlations that were statistically significantly positive in the subgroup with stage III disease were Ang-1 versus Ang-2: $r = 0.44$ ($p = 0.03$)

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

	Colon cancer	Controls	p value
Ang-1 (pg/mL)	5,227.5 (937.5-24,016.5)	5,113.5 (937.5-34,113.0)	0,913
Ang-2 (pg/mL)	3,188.0 (1,070.5-5,765.5)	1,825.5 (1,825.5-4,702.5)	0.007
Tie-2 (ng/mL)	22 (12-46)	17 (2-26)	0.036

Table 2
Plasma concentrations of Ang-1, Ang-2, and Tie-2 in stage II and stage III colon cancer patients (median, min-max).

	Stage II colon cancer	Stage III colon cancer	p value
Ang-1 (pg/mL)	5,284.5 (1,732.5-24,016.5)	5,161.5 (1,860.5-22,293.0)	0.205
Ang-2 (pg/mL)	20,84.0 (1,070.5-3,601.5)	42,92.0 (30,90.0-5,765.5)	0.003
Tie-2 (ng/mL)	22 (12-44)	23 (13-46)	0.115

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

		Ang-2		Tie-2	
		II	III	II	III
Ang-1	II	r = -0.16 (p = 0.09)		r = -0.03 (p = 0.07)	
	III		r = 0.44 (p = 0.03)		r = 0.08 (p = 0.12)
Ang-2	II			r = 0.57 (p = 0.007)	
	III				r = 0.68 (p = 0.001)

and Ang-2 *versus* Tie-2: r = 0.68 (p = 0.001), and in the subgroup with stage II disease was Ang-2 *versus* Tie-2: r = 0.57 (p = 0.007) (*table 3*).

DISCUSSION

After analysis of angiopoietin expression in tumors, the question arises as to whether there are changes in angiopoietin concentration in peripheral blood. Increased plasma concentrations of Ang-1 and Ang-2 have been observed in breast, prostate and cervical cancer [11, 15], and of Ang-2 in lung cancer [17]. An increased concentration of Tie-2 was seen in colorectal cancer [18], and was also found to be linked to metastasis [19].

In the present study, plasma concentrations of Ang-2 and Tie-2 were significantly higher in colon cancer patients than in controls, whereas there was no difference in Ang-1 concentrations between the groups.

Changes in angiopoietins and their receptor expression have been frequently observed in cancer. Results of investigations related to angiopoietin-2 expression in various tumors are unequivocal. Expression was usually increased [12, 20-22]. However, on the subject of Ang-1, opinions are controversial. For example, overexpression of Ang-1 has been observed in colorectal adenocarcinoma and breast cancer [12, 23]. Tie-2 was also overexpressed in tumors [12]. Chin *et al.* looked at serum VEGF-A, soluble VEGFR-1, soluble Tie-2 receptor, and TNF-α levels in 47 colorectal cancer patients, using quantitative ELISA prior to curative resections. Both serum TNF-α activity and sVEGFR-1 were detectable in 17% and 74% of patients, respectively. Univariate analysis demonstrated that disease-free survival was significantly associated with tumor location (p = 0.031), T category (p = 0.006), TNF-α activity (p = 0.0008), sTie-2 receptor (p = 0.012) and VEGF-A (p<0.00001). From the survival analysis, higher serum VEGF-A and sTie-2 receptor levels are associated with an earlier development of metastases. Using multivariate Cox's regression analysis, the only independent predictors of outcome were sTie-2 receptor (p = 0.038) and VEGF-A (p = 0.006) [18]. Thereafter, they also looked at the changes in serum soluble VEGFR-1 and Tie-2 receptors in colorectal cancer patients following surgical resection. In that study, 45 patients with primary colorectal cancer and 29 normal subjects were recruited. Serum VEGFR-1 and sTie-2 receptors were assayed using ELISA. sVEGFR-1 was detectable in 27% and 12.5% of cancer patients prior to curative and palliative resections, respectively, whilst 65.5% of normal controls had detectable sVEGFR-1 levels. sTie-2 receptor levels were significantly raised in patients when compared with normal controls (p = 0.0018). Furthermore, sTie-2 receptor levels were significantly higher in patients with metastases than those without (p = 0.02). sTie-2 receptors demonstrated a significant drop in patients having undergone both curative (p<0.0001) and palliative resections (p = 0.012) [19].

Ang-2 concentrations were statistically significantly higher in stage III patients than in stage II patients. The correlations between the factors studied in the whole group were weak, while in the subgroups with different stages they were stronger.

In the future, studies of these factors, using larger sample sizes and including patients at all stages of the disease should be performed to further elucidate the role of these factors in colon cancer patients and hence guide adjuvant treatment decisions.

In conclusion; Ang-1, Ang-2 and Tie-2 plasma concentrations may be valuable, additional tumor markers in colon cancer.

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