RESEARCH COMMUNICATION

Serum Granulocyte Macrophage-Colony Stimulating Factor: a Tumor Marker in Colorectal Carcinoma?

Umut Demirci[1], Ugur Coskun[1]*, Banu Sancak[2], Banu Ozturk[1], Burak Bahar[2], Mustafa Benekli[1], Suleyman Buyukberber[1]

Abstract

Background/Aims: Granulocyte macrophage colony-stimulating factor (GM-CSF) is a hematopoietic growth factor, 23 kDA molecular weight with a glycoprotein nature, which is also an immune modulator. The levels of GM-CSF and its role in the pathophysiology of several cancers such as ovarian, breast have been investigated. The aim of the present study was to determine the effect of GM-CSF and carcinoembryogenic antigen levels in predicting survival. Methodology: Plasma levels of GM-CSF were measured in 51 patients with previously untreated colorectal cancer patients and 21 healthy adults as normal controls. The clinicopathological features of colorectal carcinoma were determined at the time of blood collection. Patient staging were done according to tumor-node-metastasis (TNM) by American Joint Commission on Cancer (AJCC). Results: Plasma concentrations of GM-CSF in colorectal cancer patients (42.0 pg/ml) were statistically significant higher than normal controls (23.2 pg/ml) (p=0,001). Statistically significant correlation was not determined between pretreatment GM-CSF levels and overall survival. On the other hand, stage of disease, carcinoembryogenic antigen and peripheral leukocyte counts were not correlated with GM-CSF levels. Conclusions: This is the first report in which serum levels of GM-CSF, carcinoembriyogenic and peripheral leukocyte counts have been simultaneously evaluated in colorectal cancer patients. We found significantly elevated GM-CSF but the results suggested that serum GM-CSF may not be useful for clinical information in prognosis as a tumor marker in colorectal cancer.

Key Words: GM-CSF - colorectal cancer - tumor marker

Asian Pacific J Cancer Prev, **10**, 1021-1024

Introduction

There were diagnosed about 150,000 new cases and 50,000 individuals died annually from colorectal cancer (CRC) in the United States in 2008 (Jemal et al., 2008). The survival of CRC closely correlated with the stage of disease at diagnosis. Granulocyte macrophage colony-stimulating factor (GM-CSF) is a hematopoietic growth factor and an immune modulator, produced by monocytes, endothelial cells, fibroblasts, macrophages and T-lymphocytes (Lau et al., 1996). Its stimulators are bacterial endotoxins and inflammatory cytokines (Megyeri et al., 1990; Schwager and Jungi, 1994; Enzler et al., 2003). GM-CSF stimulates the production, proliferation, differantiation, and activation of granulocytes, macrophages, and dendritic cells (Lieschke and Burgess, 1992a; 1992b). GM-CSF receptor contains intrinsic tyrosine kinase (TK) activity triggers signaling pathways that induce cell survival. Also, GM-CSF facilates the survival of mitogen-activated protein kinase (MAPK) pathway, including ERK, p38, and JNK (Gobert Gosse et al., 2005; Himes et al., 2006; Curry et al., 2008) The levels of GM-CSF and its role in the pathophysiology of several cancers such as colorectal, ovarian, breast were investigated (Katsumata et al., 1996; Nakata et al., 1996; Scholl et al., 1996; Watanabe et al., 1998; Foti et al., 1999; Gerharz et al., 2005; Rutkowski et al., 2002; Scholl et al., 1994; Mroczko et al., 2007; Shantha Kumara et al., 2008). Although GM-CSF tumor promoting effects have been reported in several studies (Calatayud et al., 2002; Natori et al., 2002), it enhances antibody-dependent cellular cytotoxicity (ADCC) against tumor cells, the number of macrophages and their antitumoral activity (Dranoff et al., 1993, Hill et al., 1996; Ragnhanmmar, 1996). However, GM-CSF has also profound effects on the functions of leukocytes that make it especially relevant to cancer therapy (Dranoff 2004; Grabstein et al., 1986; Kubota et al., 2009). The concentration of plasma GM-CSF levels and its effects in patients with CRC have not been studied adequately. We therefore investigated the clinic importance of GM-CSF levels in the evaluation of CRC, focusing on its possible use as a tumor marker.

[1]Department of Medical Oncology, [2]Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey
**For Correspondence: ugurcos@hotmail.com*

Materials and Methods

Plasma levels of GM-CSF were measured in 51 patients in the previously untreated CRC patients and 21 healthy adults as normal controls. GM-CSF levels were analyzed in these groups. Patients whom followed-in Medical Oncology subdepartment of Gazi University Faculty of Medicine in Ankara, Turkey between January 2005 and February 2008 were enrolled into this study. The study was approved by local ethics committee according to Good Clinical Practice guidelines and each patient provided informed consent. These patients who had not received prior radiotherapy or chemotherapy, were enrolled into the study. The diagnosis of CRC was confirmed by pathological examination in all cases. Peripheral blood samples were collected from every patient prior to treatment, centrifuged to obtain serum samples and stored at -80°C until assayed. All patients with neoplasms or chronic medical illnesses were excluded from the study. The features of CRC were determined by physical examination and imaging modalities at the time of diagnosis. Cancer was staged according to tumor-node-metastasis (TNM) by American Joint Commission on Cancer (AJCC).

A preliminary statistical analysis revealed that the distribution of GM-CSF and tumor marker levels did not follow a normal distribution. Thus, the Mann-Whitney U test was used for statistical analysis. Data were presented as median and range. Statistically significant differences were defined as comparisons resulting in $p<0.05$. To analyze the associations between variables, the Kruskall–Wallis and Spearman correlation coefficients were employed.

Results

Fifty one patients (31 males, 20 females) and 21 healthy adults (12 males, 9 females) were included into the study. The patients median age wa 60 (range: 24-86) years, control group median age was 57 (range: 25-72). Among 51 patients; 4 were stage I, 17 were stage II, 7 were stage IIIb, 8 were stage IIIc and 15 were stage IV (Table 1). Statistical analysis revealed that the distribution of GM-CSF and tumor marker levels did not follow a normal distribution thus we used non–parametric tests (the Mann-Whitney U test). Serum GM-CSF levels in CRC patients (41.95pg/ml) were significantly higher when compared with control group (23.3 pg/ml) (p=0,001).

Table 1. Pretreatment Patient Characteristics

Characteristic		Number	(%)
Age		60	(range 24-86)
Sex	Male	31	(60.8)
	Female	20	(39.2)
Location	Rectum	29	(56.8)
	Colon	22	(43.2)
Tumor Stage	Stage Ib	4	(7.8)
	Stage II	17	(33.3)
	Stage IIIb	7	(13.7)
	Stage IIIc	8	(15.6)
	Stage IV	15	(29.4)

Table 2. Result of Spearman Brown Correlation Coefficient between GM-CSF and OS, CEA, WBC

Variables	N	r	P
GM-CSF and OS	51	0.12	0.41
GM-CSF and CEA	47	0.09	0.54
GM-CSF and WBC	51	0.17	0.23

Table 3. Comparison of Stage with GM-CSF (Kruskall Wallis Test)

Variable	Stage	N	Rank Average	Sd	$\chi 2$	p-value
GM-CSF	1. Stage I	4	28.88	4	3.65	0.46
Levels	2. Stage II	17	25.88			
	3. Stage IIIb	7	18.14			
	4. Stage IIIc	8	32.50			
	5. Stage IV	15	25.57			

GM-CSF levels were similar in advanced disease (stage III, IV) and early disease (stage I, II). And no significant correlation was determined between GM-CSF levels and stage of disease (c2(4)=3.65, p>0.05) with Kruskal Wallis test (Table 2). Also no significant correlation was detected between GM-CSF, OS (r=0.12, p>0.05), CEA (r=0.09, p>0.05) and peripheral leucocyte counts (r=0.17, p>0.05) with Spearman Brown correlation coefficient (Table 3).

Discussion

GM-CSF is an immune modulator with glycoprotein nature. GM-CSF has effects on mature leukocytes also it enhances neutrophil adhesion by inhibiting migration (Lieschke and Burgess, 1992a; Lieschke and Burgess 1992b) and produces hematopoietic cytokines for induction of antitumor immunity. In recent study, vaccination with irradiated B16 melanoma cells engineered to secrete murine GM-CSF that has potent and durative antitumor immunity (Dranoff et al., 1993). GM-CSF levels were determined in several cancers and were related with poor prognosis in patients with solid tumors (Foti et al., 1999; Shantha Kumara et al., 2008; Gerharz et al., 2005; Scholl et al., 1996; Rutkowski et al., 2002; Katsumata et al., 1996; Scholl et al., 1994; Mroczko et al., 2007). The GM-CSF levels in CRC patients were significantly higher when compared with those of the control group in our study (p=0,001). However no significant correlation was determined between GM-CSF and OS (r=0,12, p>.05). In recent studies, GM-CSF was elevated in breast cancer patients with advanced disease compared with localized disease (McDermott et al., 2002, Scholl et al., 1986).

With several studies, high levels of GM-CSF were detected in epithelial ovarian cancer patients. Moreover this elevation was related with unfavorable prognosis (Foti et al., 1999; Lidor et al., 1993). However we were not determined similar results that GM-CSF levels were no differences between advanced disease and early disease (p> 0.87) and no significant correlation with prognosis. Anagnostopoulos et al. (2005) showed that enhanced GM-CSF levels in CRC patient were related with eosinophil chemotactic factors. Another case was related about metastatic lung carcinoma associated with eosinophilia and production of GM-CSF (20). Similarly, Nakata et al.

(1999) described a case of thyroid cancer, eosinophilia, and high levels of GM-CSF. Although in our study no significant correlation was detected between GM-CSF and peripheral leukocyte counts, if we were performed eosinophil counting as above articles we could also detect this association.

Although in our study there was no correlation determined between GM-CSF levels and stage of disease (p>.05) in Mroczko et al's (2007) study, serum levels of GM-CSF and tumor markers were significantly higher in cancer patients compared to adenomas patients and the control group. On the other hand, they postulated the pretreatment GM-CSF level usefulness as a tumor marker for CRC, especially in combination with CEA. In the current study GM-CSF was correleted with CEA however not statistically significant.

A recent study suggested that GM-CSF deficient animals had fewer tumor metastases than animals producing normal levels of GM-CSF in breast cancer models. Based on this result, Lin et al. (2008) speculated that GM-CSF would influence myeloid cells to produce proangiogenic factors to promote tumor metastases. Similarly, Eubank et al (2009) described a mechanism by which GM-CSF promotes vascular endothelial growth factor (VEGF) production and angiogenic activity by monocytes. They suggest that GM-CSF can reduce angiogenesis and metastases in murine breast cancer. Kutoba et al (2009) showed that GM-CSF inhibition effectively suppressed tumor angiogenesis in mouse osteosarcoma. Although GM-CSF is an important factor in tumor growth, the specific mechanism of GM-CSF to promote angiogenesis and cancer metastases is not known. We think that GM-CSF inhibition can be a strategy for cancer treatment.

To our knowledge, this is the first report in which serum levels of GM-CSF, CEA and WBC have been simultaneously evaluated in CRC patients. The serum levels of GM-CSF in CRC patients were significantly higher than healthy controls, while GM-CSF did not show differences with regard to stage of disease, CEA and WBC. And we suggest that serum GM-CSF levels may not be useful for clinical information in prognosis as a tumor marker in colorectal cancer. We do not have any data about conditions and factors that regulate expression of GM-CSF in colorectal cancer patients and also with elevated patient population studies, GM-CSF's role can be evaluated as a tumor marker.

Acknowledgements

The authors declare no conflict of interest.

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