CXCL8, IL-1β and sCD200 are pro-inflammatory cytokines and their levels increase in the circulation of breast carcinoma patients

BETUL CELIK[1], ARZU DIDEM YALCIN[2,3], GIZEM ESRA GENC[4], TANGUL BULUT[1], SIBEL KULOGLU GENC[4] and SAADET GUMUSLU[4]

Departments of [1]Pathology and [2]Department of Internal Medicine, Allergy and Clinical Immunology Unit, Antalya Training and Research Hospital, 07100 Antalya, Turkey; [3]Genomics Research Center, Academia Sinica, Taipei 115, Taiwan, R.O.C.; [4]Department of Medical Biochemistry, Faculty of Medicine, Akdeniz University, 07070 Antalya, Turkey

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Abstract. The influence of biomarkers on carcinogenesis has been investigated extensively. Whether they promote carcinogenesis or work against cancer development remains to be elucidated. To the best of our knowledge, the novel molecule cluster of differentiation 200 (CD200) has not been studied on human breast cancer subjects. The present study aimed to evaluate interleukin-1β (IL-1β), C-X-C motif chemokine ligand 8 (CXCL8), cancer antigen 15.3 (CA 15.3) and the soluble CD200 (sCD200) levels in the serum samples of breast carcinoma patients in order to predict their role in breast carcinoma. The subjects included individuals with early and advanced stage breast cancers, as well as healthy controls. Commercially available ELISA kits were used to measure the serum concentrations of sCD200, IL-1β, CXCL8, CA 15.3, C-reactive protein (CRP) and leukocyte count. A total of 130 subjects were recruited; 50 early stage cancer, 50 advanced stage and 30 control subjects. Serum sCD200, CXCL8, IL-1β and CRP levels were significantly higher in the early as well as the advanced stage breast cancer patients compared to the control group. The level of CA 15.3 was statistically different between early and advanced stage. There were significant positive correlations between IL-1β and CXCL8, and IL-1β and serum sCD200 levels in the control group. These correlations did not persist in the early or the advanced stage cancer groups except CRP and CA 15.3, but new correlations appeared between serum sCD200 level and leukocyte count for advanced stage breast cancer group. Multivariate regression correlation analysis revealed positive correlation between IL-1β and sCD200; and IL-1β and CXCL8. In conclusion, sCD200, CXCL8, CA 15.3 and IL-1β are proinflammatory molecules and their levels are influenced in breast cancer patients.

Correspondence to: Dr Betul Celik, Department of Pathology, Antalya Training and Research Hospital, Varlik Mahallesi Kazim Karabekir Cad, 07100 Antalya, Turkey
E-mail: bet_celik@yahoo.com

Abbreviations: BMI, body mass index; CD200, cluster of differentiation 200; CRP, C-reactive protein; CXCL8, C-X-C motif chemokine ligand 8; DC, dendritic cell; IL, interleukin; NK, natural killer cell; sCD200, soluble CD200 antibody; TGF, transforming growth factor; TNF, tumor necrosis factor

Key words: CD200, IL-1β, CXCL8, cancer, breast, obesity

Introduction

Cancer cells transfected with C-X-C motif chemokine ligand 8 [CXCL8; also known as interleukin-8 (IL-8)] show increased cellular proliferation, cell migration and invasion based on functional assays (1). Previous studies have shown that CXCL8 and its receptors, CXCR1 and CXCR2, were significantly upregulated in colorectal cancers and acted as regulators of proliferation, angiogenesis and metastasis (2-4). Dimberg *et al* (5) reported that high plasma levels of CXCL8 tend to be correlated with distant metastasis, indicating an advanced disease stage. Thus far, the serum level of CXCL8 in patients with breast carcinoma has not been investigated extensively.

Certain cytokines lead to an influx of macrophages into adipose tissue (6,7). These recruited macrophages in turn secrete cytokines, including ILs. IL-1β is a proinflammatory cytokine against infections; however, it also has a role in the pathogenesis of cancers (8,9). Intratumoral levels of IL-1β are significantly higher in breast cancer compared to the normal adjacent breast tissue (10).

Soluble cluster of differentiation 200 (sCD200) is a cell surface membrane glycoprotein expressed on macrophages and reported to have a key role in the regulation of the immune system. sCD200 was shown to attenuate tumor necrosis factor-α (TNF-α) production *in vivo* and was associated with suppression of natural killer cells (11,12). CD200 is considered to act through its receptor to inhibit inflammation. In carcinogenesis, the overall function of sCD200 is immune evasion of tumor cells (13). The role of CD200 in breast cancer progression and metastasis has been studied in animal breast carcinoma model, where CD200 overexpression resulted in increased lymph node metastasis (14). By contrast, CD200 expression by melanocytes resulted in decreased lung metastasis (15) and decreased metastatic growth of breast

carcinoma cells (16). Thus far, sCD200 has not been studied on human breast carcinoma subjects.

The present study focused on IL-1β, CXCL8, cancer antigen 15.3 (CA 15.3) and sCD200 in breast cancer patients. Leukocyte count and C-reactive protein (CRP) levels were assessed at the same time to allow correlation analysis of these well-known proinflammatory parameters with the novel molecules.

Materials and methods

Subjects. The subjects were all females with early and advanced stage (metastatic) breast cancers and healthy controls without any known malignancy. A total of 100 subjects (50 early and 50 advanced stage) and 30 controls were recruited at the Akdeniz University Hospital. The exclusion criteria included autoimmune and rheumatological diseases, hepatitis B, hepatitis C and human immunodeficiency virus positivity. The Institutional Review Board of Akdeniz University Faculty of Medicine approved this study, and all participants provided informed consent.

Weight and blood analysis. Weight (in kilograms) was measured in light clothing, without shoes, to the nearest 0.1 kg. Subsequent to overnight fasting, blood samples were obtained from all subjects. The serum was separated by centrifugation of the blood samples at 1,500 x g for 10 min at 4˚C. The serum samples were stored at -80˚C until analytical measurements were performed.

Analysing serum levels. Commercially available ELISA kits were used to measure the serum concentrations of sCD200 (cat. no. SEK10886; SinoBiological, Inc., Beijing, China), IL-1β (cat. no. 851.610.005; Diaclone, Besançon, France), CXCL8 (cat. no. 555244; BD OptEIA kit, BD Biosciences, San Jose, CA, USA), CA 15.3 (Roche Diagnostics GmbH, Mannheim, Germany) leukocyte count (Siemens ADVIA 2120i; Siemens AG, Munich, Germany) and CRP (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's recommended protocols.

Statistical analysis. Results are presented as mean ± standard error of the mean and median ± standard deviation. Comparison of the parameters between two groups was performed using independent samples t-test. The association between the variables was determined using Pearson correlation analysis. $P<0.05$ was considered to indicate a statistical significance difference. Statistical analyses were performed using SPSS 18.0 (IBM Corp., Armonk, NY, USA).

The data were evaluated using the StataMP software package, version 12.1 (StataCorp, College Station, TX, USA) on a Mac Pro 2x2.93 GHz, 2*6 Core Intel Xeon system with 24 GB RAM using Mac OS X 10.7.4 (Apple, Cupertino, CA, USA). In general, a type I error level of $P<0.05$ was used for the statistical analysis. The Wilcoxon matched pairs signed-rank test was used to assess the differences in the variables prior to and 3 months after the onset of treatment. Correlation tables were calculated for all the variables. Furthermore, a multivariate correlation/regression analysis was applied to the data, using the survival as the dependent variable.

Results

Subject characteristics. The subjects were individuals with early and advanced stage breast cancer and healthy controls. A total of 100 subjects (50 early and 50 advanced stage cancers) and 30 healthy control were recruited. Ages ranged from 36 to 55 years (mean, 41.09, 51.71 and 51.60 years for the control, early stage cancer and advanced stage cancer groups, respectively). The characteristics of the overall study population are listed in Table I. Although not intended, all the studied patients and control groups were overweight [body mass index (BMI) ≥25 and <30 kg/m^2; n=130). Table I shows the characteristics of the control group, and the early and advanced stage breast cancer subjects. All parameters studied were increased when compared to the control. Serum sCD200, CXCL8, IL-1β and CRP levels were all significantly higher in early and advanced stage breast cancer patients (Table I). The sCD200 level of the control group was 7.96±0.37 pg/ml, while it was 10.90±0.46 pg/ml (p=0.001) and 10.60±0.40 pg/ml (p=0.001) in the early and advanced stage breast cancer groups, respectively (Table I). The CXCL8 level of the control group was 13.91±0.51 pg/ml, and 16.49±0.50 pg/ml (p=0.002) and 17.13±0.71 pg/ml (p=0.001) in the early and advanced stage breast cancer groups, respectively. The IL-1β level in the control group was 20.36±1.36 pg/ml, and it was 32.26±1.37 pg/ml (p=0.001) and 34.58±1.75 pg/ml (p=0.001) in the early and advanced stage breast cancer groups respectively. The CA 15.3 level of the control group was zero, while it was 21.00±1.77 and 28.10±2.86 IU/ml in the early and advanced stage breast cancer groups, respectively. For the CRP level, the control group had a lower level of 29.57±0.67 IU/l, while it was 41.31±0.51 IU/l (p=0.001) and 39.17±0.66 IU/l (p=0.001) in the early and advanced stage breast cancer groups, respectively. The differences in CRP between the early and advanced stage breast cancer groups were statistically significant (p=0.024).

Correlation analysis. A correlation analysis was performed to investigate whether circulating sCD200, CXCL8, CA15.3 and IL-1β levels were associated with age, BMI, CRP and leukocyte count. The level of CA 15.3 was statistically significant between the early and advanced stage cancer group (p=0.040) There were significant positive correlations between the serum IL-1β and serum sCD200 level (r=0.570, p=0.011); and IL-1β and CXCL8 (r=0.779, p=0.001) within the control group. These correlations did not persist within the early or advanced stage cancer groups; however, new correlations appeared between the serum sCD200 level and leukocyte count (r=0.546, p=0.009) for the advanced stage breast cancer group. The advanced breast cancer group also revealed a positive correlation between CRP and CA 15.3 (r=0.410, p=0.013).

Subsequent to adjusting for the early and advanced stage breast cancer groups, the correlation between sCD200 and leukocyte count (r=0.359, p=0.021) and CRP and CA15.3 (r=0.264, p=0.032) remained significant. Multivariate regression correlation analysis revealed a positive correlation between IL-1β and CD200 (r=0.570, p=0.011); and IL-1β and CXCL8 (r=0.810, p=0.001), which were observed in the univariate analysis for control group.

Table I. Patient characteristics and biomarkers levels in the control, early stage and late stage breast cancer subjects.

Parameter	Control (n=30)	Early stage (n=50)	P-value[a]	Late stage (n=50)	P-value[b]	P-value[c]
Age, years	41.09±1.97	51.71±1.48	NA	51.60±1.57	NA	NA
BMI, kg/m^2	26.07±1.12	29.47±0.73	NA	28.96±0.72	NA	NA
sCD200, pg/ml	7.96±0.37	10.90±0.46	0.001	10.60±0.40	0.001	NA
IL-1β, pg/ml	20.36±1.36	32.26±1.37	0.001	34.58±1.75	0.001	NA
CXCL8, pg/ml	13.91±0.51	16.49±0.50	0.002	17.13±0.71	0.001	NA
CRP, IU/l	29.57±0.67	41.31±0.51	0.001	39.17±0.66	0.001	0.024
CA 15.3, IU/ml	0.00	21.00±1.77	NA	28.10±2.86	NA	0.040
Leukocytes, 1,000/mm^3	8.48±0.41	7.67±0.30	NA	7.25±0.37	NA	Naat clean

BMI, body mass index; sCD200, soluble cluster of differentiation 200; IL, interleukin; CXCL8, C-X-C motif chemokine ligand 8; CRP, C-reactive protein; CA 15.5, cancer antigen 15.3; NA, not available; [a]control vs. early stage cancer; [b]control vs. late stage cancer; [c]early vs. late stage cancer.

Discussion

An association between the development of cancer and inflammation has repeatedly been reported (17-19). Overexpression of CXCL8, an angiogenic factor, by tumor cells is well known (20) and its level was correlated with breast and colon cancer progression (21-24). IL-1β is another proinflammatory molecule. It is present in the extracellular space and its level is undetectable when there is no inflammation. IL-1β-deficient mice experienced slow tumor progression (25,26). It appears that inflammation provides an environment for tumor cells. Its presence in breast cancer was correlated with aggressiveness of the tumor (27,28). These studies were conducted on breast cancer tissues *in vitro* and the involvement of the circulatory system has not been investigated previously. The present results represented the circulating level of these cytokines and higher levels of CXCL8 and IL-1β were identified in the serum of breast cancer patients compared to the control group. The study also showed that there was an increased level of CRP in the serum of breast cancer patients when compared to the control group, which indicates a proinflammatory state among breast cancer patients.

The importance of the inflammatory responses in determining disease progression in patients with cancer is recognized. In particular, white cell counts (neutrophil), platelet counts and acute-phase proteins have been reported to have a prognostic value. CRP is a classical acute-phase reactant protein from the pentraxin family. CRP is increased in the circulation in response to acute inflammation, infection and tissue damage. Studies reporting a higher level of CRP in breast carcinoma cells claimed that elevated CRP levels were associated with an increased risk of breast cancer (29) or had a prognostic value in patients with breast cancer (30). By contrast, the present study indicates that the cancer state in the body increases CRP levels to fight against tumor cells. CXCL8 and IL-1β are there as part of an inflammatory response that is fighting against tumor.

One aspect of the present study was that there was a positive correlation between IL-1β and CD200, and IL-1β and CXCL8 in the overweight control group. There is a low-grade chronic inflammation state in obese people and serum CRP and CXCL8 levels are increased (31,32). However, the groups in the present study consisted of overweight subjects, not obese. Adipose tissue functions as an endocrine organ and secretes bioactive molecules (33,34), and several epidemiological studies have established a consistent association between obesity and cancer (31). One study found a positive association between CRP levels and postmenopausal breast cancer risk, which was restricted to women with excess adiposity (35). Considering the association of CXCL8 with obesity (36), adipose tissue in the breast parenchyma may affect cytokine levels.

Studies have reported that the CD200/CD200-receptor interaction has an anti-inflammatory effect (37,38). It increases the anti-inflammatory cytokines IL-10, IL-4, IL-5, TGF-β and regulatory T cells, decreases IL-1, IL-2, IL-6, IL-12, CTL, TNF-α and leukocytes, and increases tumor progression (39-41). Genetically engineered mice that overexpressed CD200 showed that the metastatic growth of breast tumor cells increased (14). By contrast, highly aggressive, but similar, breast carcinoma cells had decreased metastatic growth in a CD200-overexpressed host (16). These two opposing effects may be explained by the bidirectional characteristic of CD200. However, the present study observed higher sCD200 level in breast carcinoma patients compared to the control group. Its level was positively correlated with proinflammatory cytokine IL-1β in the control group and the leukocyte count in the advanced stage breast carcinoma group. These results indicate the proinflammatory effects of this cytokine. Its proinflammatory effect has been detected in our previous studies conducted on allergic patients (42,43). We hypothesize that a decreased metastatic growth in a CD200-overexpressed host (16) reflects its proinflammatory property and a higher sCD200 level observed in the cancer group is the indication of the host reaction against cancer development in the present study. A higher level observed in the tumor does not reflect its anti-inflammatory or tumorigenic property, but the inflammatory reaction of the host against tumor development. In a previous study (15), the activation of the CD200 receptor, which is expressed mostly by myeloid cells (44), may have resulted in less CD200-negative melanoma cells due to the antigen masking effort of the tumors to escape immune surveillance.

The correlation that was apparent between CRP and CA 15.3 in the present study is important. Previously, a significant decrease in CA 15.3 was observed following immunotherapy for breast cancer (45). Furthermore, the correlation between CA 15.3 and CRP has been documented in different types of cancer patients after the inhibition of pro-inflammatory pathways by vitamin C (46). To the best of our knowledge, this is the first study demonstrating such a correlation in breast cancer.

In conclusion, sCD200, CXCL8, IL-1β and CRP are proinflammatory molecules and their levels are influenced in breast cancer. Further studies comparing obese (BMI ≥30 kg/m^2) and non-obese breast carcinoma subjects may allow novel insight regarding obesity, cancer and inflammatory markers.

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