EXPRESSION AND CLINICAL SIGNIFICANCE OF CD44 IN THE PERIPHERAL BLOOD OF PATIENTS WITH CASTRIC CANCER

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ABSTRACT

Objective: To investigate the clinical significance of cell adhesive molecule (CD44) expression on periphery blood (PB) of patients with gastric cancer. Methods: Both the level of CD44 and the immunocyte phenotype of the lymphocytes of 110 patients with gastric cancer and 100 healthy subjects were examined by flow cytometry, and the results were analyzed pathologically and statistically. Results: The mean of the CD44% in PB of the healthy subjects was 46.14±13.4 and there were no statistic differences for their age and sex. Site of tumor growth: The significant difference (P<0.01) was present between the patients with the cardiac part (C) or body of gastric (M+C) cancer and normal individuals. The significant difference (P<0.05) was present between patients with the 3 sites involved gastric cancer and normal individuals. Type of tumor mass: The significant difference (P<0.05) was present between patients with mixed type gastric cancer and normal individuals. Size of tumor mass: The significant difference was present between the normal individuals and patients with gastric cancer > 10 cm mass (P<0.01) and 7~10 cm mass (P<0.05). Degree of tumor differentiation: The significant difference (P<0.01) was present between the patients with low differentiation gastric cancer and normal individuals. The significant difference (P<0.01) was present between patients with metastatic stage: The significant difference (P<0.01) was present lymph node.

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In recent years it was noted that the levels of adhesive molecules (CD44) in patients with malignancy were related to metastasis of tumors.1-6 But only the expression of CD44 on malignant tumor cells was reported in medical literature. Up to now few reports appeared on the examination of CD44 level by flow cytometry in PB of patients with gastric cancer. In this work we investigated CD44% both in 110 patients with gastric cancer before operation and in 100 healthy subjects, and analyzed the clinical and pathological status of patients.

MATERIALS AND METHODS

Patients and Control Group

The study included 110 patients with gastric cancer before operation hospitalized in the surgery department of Zhejiang Cancer Hospital from June 1997 to July 1998.
All their pathological diagnosis and medical records data were reexamined. 100 healthy adult volunteers were from the hospital staff with mean age of 38.7 years (ranging 19–66).

**Agents**

All the antibodies used in the study are fluorescein isothiocyanate (FITC)-labeled or phycoerythrin (PE)-labeled mouse antihuman monoclonal antibodies. They are CD19-FITC, CD56 (NK cells)-PE, CD19 (B cells)-FITC (commercial kits of Becton Dickinson) and CD3 (T cells)-FITC, CD4 (Helper T cells)-FITC, CD8 (Suppressor T cells)-PE (commercial kits of Immunity Department of Beijing Medical University).

**Methods**

2 ml peripheral blood samples from each case or healthy subject were put into the tubes containing 2 ml anticoagulant (25 μl/ml heparin). 5 plastic tubes (12 mm 75 mm) were prepared for each case or healthy subject. and 20 μl of antibody were added to each tube as follows: tube A CD19-FITC; tube B CD3-FITC/CD8-PE; tube C CD3-FITC/CD56-PE and tube D CD44-FITC. Then 100 μl anticoagulant blood was added to each tube and mixed thoroughly with the antibodies. Tube E served as a negative control tube. All tubes were incubated at room temperature for 15–30 min in the dark. Then 2 ml globulolytic solution was put into each tube to remove erythrocytes by lysis. Following 10–12 min incubation at room temperature in the dark, the tubes were centrifuged (300 g) for 5 min and supernants were remove. The remained cells in each tube were washed twice with 2 ml PBS (containing 0.1% sodium azide). The washed cells were resuspended in 0.5 ml of cell solution and analyzed immediately for fluorescence on the FACSCalibur flow cytometer (Becton Dickinson). For each tube the flow cytometer collected more than 2000 cells within the lymphocyte gate. The percentage of positive labeled lymphocytes were analyzed using cell guest software (Becton Dickinson). For the analyses of CD44 and Phenotype of lymphocytes, histogram (Figure 1, 2) and dotgram were used respectively.

**Statistics**

The student's 't' test was used for statistical analysis.

**RESULTS**

**The Range of CD44% in PB from Healthy Subjects**

The mean of CD44% in PB from healthy subjects was 46.14±13.4.

**Sites of Tumor Growth**

All of the 110 gastric cancer patients had sites of tumor growth in their medical records. Table 1 shows the significant difference of CD44% between healthy subjects and patients with different sites of gastric cancer.

**Macropathology Type of Gastric Cancer**

All of the 110 gastric cancer patients had 102 macropathology types in their medical records. Table 2 shows the significant difference of CD44% between healthy subjects and patients with different macropathology types of gastric cancer.

**Size of Gastric Cancer**

All of 108 patients with gastric cancer had medical records of tumor size. Table 3 shows that there was significant difference of CD44% between healthy subjects and patients with different size of gastric cancer.

**Differentiation Degree of Gastric Cancer**

All cases had pathology diagnoses. The significant difference of CD44% between gastric cancer patients with various differentiation degrees and healthy subjects was shown in Table 4.

**Lymph Node Metastasis Status**

All the patients with gastric cancer had lymph node metastasis of tumor in their medical records. Table 5 shows that the CD44% in gastric cancer patients with or without lymph node metastasis were significantly higher than that in healthy subjects.

**Clinic Staging**

The CD44% of patients with gastric cancer in various clinic stages were shown in Table 6 and were significantly higher compared with healthy subjects.

**The Ages of Patients**

The CD44% in the patients were significant higher than that in healthy subjects in the same age groups (Table 7).

**The Sexes of Patients**

All the patients with gastric cancer without statistic differences for their sexes.