



# Expression of E-selectin, integrin $\beta_1$ and immunoglobulin superfamily member in human gastric carcinoma cells and its clinicopathologic significance

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## Abstract

**AIM:** To study the expression levels of E-selectin, integrin  $\beta_1$  and immunoglobulin superfamily member-intercellular adhesion molecule-1 (ICAM-1) in human gastric carcinoma cells, and to explore the relationship between these three kinds of cell adhesion molecules and gastric carcinoma.

**METHODS:** The serum contents of E-selectin, integrin  $\beta_1$  and ICAM-1 were detected by enzyme-linked immunosorbent assay (ELISA), in 47 healthy individuals (control group) and in 57 patients with gastric carcinoma (gastric carcinoma group) respectively prior to operation and 7 d after operation.

**RESULTS:** The serum E-selectin, ICAM-1 and integrin  $\beta_1$  were found to be expressed in both control and gastric carcinoma groups. However, they were highly expressed in patients with gastric carcinoma patients before operation or with unresectable tumours. The expression levels of ICAM-1 and integrin  $\beta_1$  were significantly higher in gastric carcinoma patients than in controls ( $P < 0.01$ ). A comparison of the E-selectin levels between the two groups showed statistically insignificant difference ( $P = 0.64$ ). In addition, the expression levels were all decreased substantially in the postoperative patients subjected to radical resection of the tumours, indicating that the high level expressions of these compounds might be the important factor for predicting the prognosis of these patients.

**CONCLUSION:** Serum E-selectin, ICAM-1 and integrin  $\beta_1$  expression levels are probably related to the metastasis and relapse of gastric cancer.

## INTRODUCTION

The adhesion between endothelial cells and cancer cells is the essential intermediate link for tumor metastasis mediated by specific cellular surface receptors including selectin, integrin and cell adhesion molecule family<sup>[1]</sup>. Many investigations indicate that adhesion family members are involved in the progression of cancer<sup>[2]</sup>. The binding of cancer cells to endothelial cells mediated by the E-selectin is reportedly related to their metastatic potentials<sup>[3]</sup>. The intercellular adhesion molecule-1 (ICAM-1) a member of the immunoglobulin superfamily, its ligand is the adhesion molecule (LFA-1) of the immunoglobulin superfamily. Integrin- $\beta_1$  is a member of the adhesion superfamily, which not only mediates the adhesion between cells and extracellular matrix, but also mediates the adhesion between leucocytes and blood vessel endothelial cells. The expression of E-selectin, ICAM-1 and integrin  $\beta_1$  on small blood vessels in tumour-infiltrating area at a high level is capable of suggesting the interaction between endothelial cells<sup>[4]</sup>. In the present study, we detected the serum E-selectin, ICAM-1 and integrin  $\beta_1$  contents in patients with gastric carcinoma in order to evaluate their clinicopathologic significance.

## MATERIALS AND METHODS

### Patients and healthy individuals

A total of 57 patients with gastric carcinoma, including 31 males and 26 females, at the age of 34-77 years, with a median age of 55 years, in Zhejiang Provincial People's Hospital and First People's Hospital of Taizhou were diagnosed as gastric carcinoma by pathological examination from January 2000 to December 2003. According to the WHO staging standard, 13 patients were classified into stage I, 5 patients stage II, 31 patients stage III, and 8 patients stage IV. These patients could also be

classified into intestine type and diffuse type based on Lauren's histological typing principle, and classified into well-, moderately- and poorly- differentiated types based on the predominant differentiation mode, respectively. All patients did not receive radiotherapy, chemotherapy, blood transfusion, steroid and opium drugs prior to operation. All the patients were followed up for 9 mo (1-48 mo) and their dates and causes of death were recorded.

Forty-seven healthy individuals, consisting of 31 males and 16 females, (at the age of 32-68 years, with a medium age of 51), were recruited as the control group. They were selected as blood donors and did not have any disease.

### Assay procedure

The healthy individuals and patients were all fasted overnight. Blood samples were collected from their peripheral veins at 8-9 h AM next day, and then the blood samples were stored at -80°C after the sera were separated by centrifugation. The blood samples from the patients with gastric carcinoma were collected both before and 7 d after operation respectively.

Assays for serum E-selectin, ICAM-1 and integrin  $\beta_1$  were carried out using the solid phase ELISA test kit provided by the Parameter R & D Systems (USA), following the manufacturer's instructions. The test sensitivities were estimated to be 1 ng/mL, 2 ng/mL and 0.3 ng/mL for E-selectin, integrin  $\beta_1$  and ICAM-1, respectively. Any resulting values for the patients which were 95% higher than those for the control were defined as the elevated contents of serum adhesion molecules. The cut-off values of E-selectin, ICAM-1 and integrin  $\beta_1$  were 50.4 ng/mL, 337 ng/mL and 5.2  $\mu$ g/mL, respectively. Therefore, if the cut-off values were higher than the above values, the high level expression should be considered as positive.

### Statistical analysis

All data were analyzed by the SPSS 10.0 statistical software package, and their abnormal distributions were distinguished from the normal ones. At the same time, the variability of the medium values and the distributive ranges for the experimental data were evaluated using the univariate analysis. The non-matched and matched data were evaluated using the variate Kruskal-Wallis analysis (analysis of variance, ANOVA), Mann-Whitney *U* test and Wilcoxon grade-related test. The multivariate analysis was conducted using Cox Proportional Hazards Regression Mode after the predicted variability was determined using the single factor analysis.

## RESULTS

### Contents of E-selectin, integrin $\beta_1$ and ICAM-1 and their comparison

The E-selectin, integrin  $\beta_1$  and ICAM-1 were detected in all serum samples. The content of serum integrin  $\beta_1$  in the preoperative gastric carcinoma group and control group was 4.8  $\mu$ g/mL and 2.1  $\mu$ g/mL, respectively ( $P = 0.00002$ ). The content of ICAM-1 in the preoperative gastric carcinoma group and control group were estimated to be 271 ng/mL and 193 ng/mL, respectively ( $P = 0.0004$ ). The content of E-selectin in the preoperative gastric

**Table 1** Diagnostic value of elevated E-selectin, ICAM-1 and integrin  $\beta_1$  levels in prediction of gastric carcinoma

Diagnostic value	E-selectin (> 50.4 ng/mL)	ICAM-1 (> 337 ng/mL)	Integrin $\beta_1$ (> 5.2 $\mu$ g/mL)
Sensitivity (%)	24.6	33.3	28.1
Specificity (%)	95.7	95.7	95.7
Positive prediction value (%)	87.5	90.5	88.9
Negative prediction value (%)	51.3	54.2	52.3
Predominance ratio	7.32	11.25	8.78
95% confidence region	1.57-34.15	2.46-51.42	1.9-40.73

carcinoma group and control group was 42.1 ng/mL and 39.6 ng/mL, respectively ( $P = 0.64$ ).

### Expression levels of E-selectin, ICAM-1 and integrin $\beta_1$ in gastric carcinoma patients

The positive rates of E-selectin, ICAM-1 and integrin  $\beta_1$  expression in gastric carcinoma group were 25.0%, 32.7% and 28.8%, respectively. The accuracy for diagnosing gastric carcinoma based on the expressions of these 3 adhesion molecules is shown in Table 1.

In gastric carcinoma group, 38 patients died in the progressive stage of gastric carcinoma, and tumours relapsed in 3 of the 19 remaining survivors. The serum E-selectin, ICAM-1 and integrin  $\beta_1$  levels were higher in the progressive stage of tumour, and the elevated levels were significantly correlated with the tumour staging ( $P < 0.05$ ).

No correlations were found between tumour T staging and serum E-selectin ( $P = 0.053$ ) and ICAM-1 ( $P = 0.1$ ) level. The serum E-selectin level [47.5 (36.8-58.3) ng/mL] in the patients with T4 tumour, was significantly higher than that [33.8 (28.4-41) ng/mL] of the patients with T<sub>1-3</sub> tumour ( $P = 0.006$ ). Furthermore, the serum ICAM-1 level [292 (201-437) ng/mL] in the patients with T<sub>3</sub> and T<sub>4</sub> tumour-infiltrating serous membrane layer was significantly higher than that [231 (154-266) ng/mL] of the patients with T<sub>1</sub> and T<sub>2</sub> tumours in mucous membrane, submucous layer and proper muscular layer ( $P = 0.023$ ).

The serum expression levels of E-selectin, ICAM-1 and integrin in patients with lymph node metastasis were higher than those in patients with no lymph node metastasis (the former *vs* the latter:  $P = 0.04$ , 0.004, and 0.0018, respectively). As compared with the non-remote metastatic group, the remote metastatic group had higher serum E-selectin, ICAM-1 and integrin  $\beta_1$  levels ( $P < 0.05$ ). The serum E-selectin, ICAM-1 and integrin  $\beta_1$  levels were not correlated with the tumour location (gastric antrum, gastric body or gastric cardia), tumour typing and differentiation degree (Table 2).

### Effect of surgery on expression of E-selectin, ICAM-1 and integrin $\beta_1$

Among the 57 patients with gastric carcinoma, 41 were subjected to radical gastric resection and regional lymph node cleaning, 16 had unresectable tumours. All the patients had no postoperative complications. The postoperative serum E-selectin, ICAM-1 and integrin

**Table 2** Variability analysis of the prognoses and predictive factors in patients with gastric carcinoma

Variables	Risk ratio	95% confidence region	P
Age	0.97	0.94-1.01	0.2
Sex	0.77	0.39-1.54	0.47
Tumor location (gastric antrum, gastric body or gastric cardia)	0.95	0.62-1.45	0.81
Tumour histology (intestine type and diffusion type)	1.32	0.70-2.52	0.38
Tumour differentiation degree (high, moderate, low)	1.19	0.71-1.96	0.49
Tumour staging (stages I-IV)	2.65	1.71-4.11	0.000
T (T <sub>1</sub> -4)	2.75	1.75-4.34	0.000
N (N <sub>0</sub> , N <sub>1</sub> )	4.33	1.65-11.36	0.003
M (M <sub>0</sub> , M <sub>1</sub> )	7.36	2.98-18.2	0.000
E-selectin (elevated, normal)	2.81	1.40-5.63	0.003
ICAM-1 (elevated, normal)	2.71	1.37-5.36	0.004
Integrin $\beta_1$ (elevated, normal)	2.1	1.05-4.18	0.035

$\beta_1$  contents decreased significantly ( $P = 0.04, 0.01$ , and  $0.001$ , respectively). Conversely, the postoperative serum E-selectin, ICAM-1 and integrin  $\beta_1$  levels in the 16 patients with nonresectable tumours were similar to those before operation ( $P = 0.08, 0.09$ , and  $0.2$ , respectively).

#### Variability analysis of prognosis and predictive factors in patients with gastric carcinoma

The monovariate analysis revealed that the TNM staging, tumour-infiltrating depth in gastroparietes (T status), lymph node metastasis, remote metastasis, as well as the preoperative serum E-selectin, ICAM-1 and integrin  $\beta_1$  levels were the important factors affecting the total survival rate of the patients (Table 2). The multiple statistical analysis of all the factors was carried out, the results indicated that tumour staging was the only independent factor for predicting the survival of the patients.

## DISCUSSION

The present study demonstrated that the preoperative high levels of ICAM-1, integrin  $\beta_1$  and E-selectin in patients with gastric carcinoma had higher specificity and lower sensitivity for the diagnosis of gastric carcinoma. The serum contents of the three kinds of adhesion molecules significantly correlated with tumour staging, gastropariete infiltrating, lymph node metastasis and remote metastasis. Although the relationship between serum ICAM-1 and metastasis in the present study further proved the previous observations, the correlation between the serum E-selectin and integrin  $\beta_1$  contents and remote metastasis is reportedly contradictory sometimes<sup>[5-8]</sup>.

Previous investigations showed that certain kinds of cytokines are able to induce the expression of E-selectin, ICAM-1 and integrin  $\beta_1$ <sup>[9,10]</sup>. Monocytes are the only origin of sICAM-1. Large amounts of sICAM-1 in serum could be found in the culture of certain tumour cell strains' indicating that tumour cells may also be the origin of sICAM-1 which may explain the decreased serum sICAM-1 in patients with gastric carcinoma after resecting

of their tumours. Moreover, sICAM-1 could not be produced through different forms of mRNA splicing<sup>[8,10]</sup>.

In the present study, all the preoperative serum E-selectin, ICAM-1 and integrin  $\beta_1$  levels in the patients with gastric carcinoma were found to be an important factor affecting the prognosis of the patients, suggesting that the levels of E-selectin, ICAM-1 and integrin  $\beta_1$  can be used as supplementary markers to determine the disease condition, stage and prognosis, as well as the therapeutical efficacy in the patients. However, multiple statistical analyses of all the factors have revealed that tumor staging could be used to predict the survival of cancer patients as an independent factor. The discrepancy between effects of the adhesion molecule levels in peripheral circulation on the prognosis of patients with gastric carcinoma in different reports is possibly attributed to the different number of patients used and the different progression stage of the disease<sup>[9-11]</sup>.

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