Determinations of Cell Phenotypes CD49b、CD49d of Peripheral Blood Lymphocytes in Patients with Colorectal Cancer

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Abstract **Objective** To investigate the expression of CD49b and CD49d in peripheral blood lymphocytes in patients with colorectal cancer before and after surgery. **Methods** The two kinds of cell phenotypes were measured by flow cytometry in 35 cases of patients with colorectal cancer. **Results** The expression of CD49b and CD49d in cancer group was much lower than those in control group, in Dukes D stage group was significantly lower than those in Dukes B stage group, in lymph node metastasis group was much lower than those in lymph node negative group. The expression of CD49d in serosa involved group was lower than that in no serosa involved group. After radical or palliative resection of the tumor, CD49b was increased significantly. CD49d was increased significantly in radical resection group, while no significant change was observed in palliative resection group. **Conclusion** Cell phenotypes of CD49b and CD49d of peripheral blood lymphocytes in patients with colorectal cancer are closely related to biological characteristics of the tumor. Removal of the tumor may be benefit to improve the cellular immunity of the patients.

Key Words Colorectal neoplasm; Lymphocyte; Phenotype; Cytometry

The process of tumor development and progression are closely correlated with the patients´ immune condition. Cellular immunity is the major manner against the tumor. Base on our previous studies on the lymphocyte phenotype in patients with gastric cancer[1], we determined the pre and postoperative peripheral blood lymphocyte phenotype CD49b, CD49d by using monoclonal antibody against CD49b、CD49d and flow cytometry in 35 patients with colorectal cancer, and thereby explore the relationship between the peri-operative immune condition and the tumor biologic behavior.

MATERIALS AND METHODS

Clinical materials

Of 35 colorectal cancer patients (17 male and 18 female, age ranged 25~80 years old and average 59 years old) recruited in this study, 18 cases were diagnosed as colonic cancer and the rest 18 were rectal cancer. All diagnosis was confirmed intra-operatively and pathologically. Dukes stage: 10 cases were in Dukes B, 12 cases Dukes C and 13 cases Dukes D. Histological type: 15 were adenocarcinoma, 4 were poor differentiated adenocarcinoma and 1 was mucinous adenocarcinoma. Invasive depth: 15 patients without serosa invasion and 20 with the invasion. Twenty five patients had lymph nodes metastasis and 10 patients didn´t.

Control group was composed of 30 patients without any neoplasias in the same time. Among them, 19 male and 16 female, aged 22~81 (average 56) years old. Diagnosis: 17 had inguinal hernia, 5 great saphenous varication, 4 anal fistula and 4 hemorrhoids. All controls had no medical conditions which may affect the lymphocyte phenotypes, such as malignancies, autoimmune diseases or inflammatory diseases, etc.

Reagents and laboratory assay

All monoclonal antibodies were mice IgG against human antigens. Anti CD49b, CD49d antibodies were purchased from Coulter-Immunotech, Inc, USA. Three days before the surgery, peripheral venous blood sample was drawn from the patients in both groups and collected in a heparin-coated tube. For the patient group, the blood samples were also collected 10 days after the surgery. The method used for analysis was previously described by Li Li et al.[2] Briefly, 100 μl blood and 10 μl Coulter monoclonal antibody with directly labeled fluorescence was added into a 12 mm × 75 mm tube, mixed and incubated at room temperature for 15 minutes. The sample then was treated with Q-PREP and analyzed on flow cytometry (EPICS-XL).

Statistic analysis

paired or unpaired student t test and ANOVA test.

RESULTS

The expression of lymphocyte phenotype CD49b, CD49d in patients with colorectal cancer (Table 1).

Both CD49b and CD49d were significantly lower in patient group than that in controls (p<0.05 and p<0.01 respectively.

The relationship of the expression of lymphocyte phenotype CD49b, CD49d to the stage, lymph nodes metastasis and invasive depth of colorectal cancer (Table 2).

The expression of CD49b, CD49d was significantly lower in Dukes D patients than in Dukes B patients (p<0.05); In patients with lymph nodes metastasis the expression of CD49b, CD49d was also significantly lower as compared to those without positive lymph node (p<0.05); Further more, the expression of CD49d in the patients with serosa invasion was lower than that in those patients with-

Table 1. Expression of CD49b and CD49d in patient group and controls ($\bar{x}$±s, %)

Groups	Number	CD49b	CD49d
Controls	30	33.18±17.18	83.61±9.14
Patients	35	22.77±10.46*	65.59±13.15**

* P<0.05, ** P<0.01, as compared with controls.

Table 2. The expression of CD49b、CD49d in patients with different clinical pathological characteristics ($\bar{x}$±s ,%)

Group	Number	CD49b	CD49d
Dukes Stage			
Dukes B	10	29.60±11.01	73.12±10.86
Dukes C	12	21.66±8.58	66.62±7.83
Dukes D	13	18.53±9.59*	59.85±16.18*
Lymph nodes metastasis			
Negative	10	29.60±11.01	73.12±10.86
Positive	25	20.03±9.07△	63.19±13.10△
Sarosa invasion			
Negative	15	24.80±11.05	71.34±12.88
Positive	20	21.24±10.01	61.32±11.95▲

* P<0.05 as compared with Dukes B patients;
△ P<0.05 as compared with the patients without positive lymph nodes;
▲ P<0.05 as compared with the patients with negative sarosa invasion.

Table 3. The influence of radical and palliative resection of the tumor on the expression of CD49b and CD49d($\bar{x}$±s,%)

	Number	CD49b	CD49d
Radical resection			
Preoperative	24	24.01±12.18	67.37±12.53
Postoperative	24	32.18±13.79**	72.15±12.25*
Palliative resection			
Preoperative	11	20.06±4.35	61.72±14.23
Postoperative	11	29.56±11.30△	65.23±12.76

* P<0.05, ** P<0.01, as compared with that before radical resection of the tumor;
△ P<0.05, as compared with that before palliative resection of the tumor.

out the serosa invasion ($p<0.05$).

The pre and post operative expression of CD49b, CD49d in patients with colorectal cancer (Table 3).

The expression of CD49b was significantly elevated after the radical ($p<0.01$) or palliative ($p<0.05$) resection of the tumor. In contrast, the expression of CD49d only significantly increased after the radical resection of the tumor ($p<0.05$), but not changed after the palliative resection ($p>0.05$).

DISCUSSIONS

As we know, T cell plays very important role in the anti-tumor immune function. Recent studies have shown that T cell function is deficient in patients with malignant tumor, which closely associates with the tumor burden. Variations of CD3、CD4、and CD8 have been frequently used as observing parameters in the majority of previous studies on T cell function, however studies on the changes of CD49b、CD49d are relatively fewer. The adhesive molecules are a large group of glucoprotein or glucolipid, which act through the interaction between the ligand and receptor. These molecules are broadly distributed on the cell surface and mediate the cell-to-cell, cell to matrix, and cell to matrix to cell adhesions. They are involved in a serials of physiological or pathophysilogical responses such as the signal transfer, migration, growth and differentiation of the cells and oncogenesis, trauma healing, etc[3]. CD49b、CD49d are the very late antigens belong to the family of intergrin. They are the ligands of collagen, leminin and fibronectin. They mediate the adhesion between cell and its matrix, and between the white cell and vessel endothelial cells [4,5]. Our previous studies have revealed that the type IV collagen and laminin were significantly elevated in the peripheral blood of the patients with colorectal cancer, and associated with the biological characteristics of the tumor[6], the variation of their ligands, however, remained to be further clarified. The result of present study has shown the expression of CD49b、CD49d was significantly reduced in patients with colorectal cancer as compared with the controls, it was notably lower in the patients with dukes D stage tumor than those in the patients with Dukes B tumor. It was also lower in the patients with lymph nodes metastasis then that in the patients with no lymph nodes metastasis and in the patients with serosa invasion than that in the patients without. Those findings indicate that not only the level of adhesive molecules changed in the peripheral blood of the patients with colorectal cancer, but also the expression of their ligands located on the surface of lymphocyte was significantly decreased and moreover correlated with the progress of the tumor. The lower expression level of the adhesive molecular ligands on the lymphocyte surface can reduce the abilities of lymphocyte in locating and migrating to, and adhere with the tumor cells, and thereby reduce the cytotoxicity of the lymphocyte. It enhances the deficiency of immune function in cancer patients. As revealed in this study, the remarkable post-operative elevation of CD49b、CD49d expression may be helpful in improving the lymphocyte functions of locating and migrating to, adhering with, and thereby killing the tumor cells. Finally, this elevation may play some role in promoting the anti-tumor immune function in cancer patients.

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