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• BRIEF REPORTS •

Effect of P-selectin monoclonal antibody on metastasis of gastric cancer and immune function

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Abstract

AIM: To investigate the effect of cell adhesion molecule P-selectin monoclonal antibody (Mab) on metastasis and immune function of mice orthototopically implanted with human gastric cancer tissue.

METHODS: SCID mice were implanted orthotopically with SGC-7901 human gastric carcinoma tissue. Starting from day 3 after operation, animals were given intravenously PBS or P-selectin Mab (100 μg/injection) (for both normal mice and tumor-implanted mice with tumors), twice weekly for 3 weeks. Two animals in each group were sacrificed randomly at the 1st, 2nd, 4th week and 6th week. While T cell and B cell transformation indices were determined with the ^{3}H TdR infiltration method, the NK cell activity was detected by the LDH release method.

RESULTS: The metastatic rate in the P-selectin Mab treated group was lower than that in the PBS treated group (with tumors). The NK activity of normal mice increased over time. The immune functions (T, B cell function, NK activity) of the tumor group in the 6th week were significantly lower than those in the 4th week, but the change was attenuated by P-selectin Mab.

CONCLUSION: P-selectin Mab could suppress the metastasis of gastric cancer with no adverse effect on host immune function.

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INTRODUCTION

Gastric carcinoma is one of the most frequent tumors in China. Tumor metastasis is very common clinically. Cell adhesion molecules have been implicated to be crucial elements in the process of metastasis[1-28]. P-selectin is an adhesion molecules that mediates the cell to cell interaction of platelets and endothelial cells with neutrophils and monocytes as well as tumor cells[29]. Our previous study indicates that P-selectin expression is related to aggressive behavior, dissemination and poor prognosis of human gastric carcinomas, and P-selectin monoclonal artibody can inhibit gastric carcinoma metastasis[30-32]. The present study was performed to investigate effects of P-selectin monoclonal antibody on metastasis and immune function in SCID mouse metastaic models of human gastric cancer constructed by orthotopic implantation of histologically intact tumor tissue.

MATERIALS AND METHODS

Animal model

Forty-eight male SCID mice obtained from Shanghai Cancer Institute were 7-8 weeks old with weight of 20-25 g. Human gastric cancer SGC-7901, a poorly-differentiated adenocarcinoma line, was originally derived from a primary tumor and maintained by passage in nude mice subcutaneously.

SCID mice were randomly divided into experimental group (n=24) and normal group (n=24). Animal models in experimental group were made using orthotopic implantation of histologically intact tissue of human gastric carcinoma[33]. Tumors were resected aseptically. Necrotic tumor tissues were removed and the remaining non-necrotic tumor tissues were minced into pieces about 5-7 mm in diameter in Hank's balanced salt solution. Each of the tumor pieces was weighed and adjusted to be 150 mg with scissors. Mice were anesthetized with 4.3 % trichloraldehyde hydrate and an incision was made through the left upper abdominal pararectal line and peritoneal cavity was carefully exposed and a part of the serosal membrane in the middle of the greater curvature of the glandular stomach was mechanically injured by using scissors. A tumor piece of 150 mg was fixed on each injured site of the serosal surface. The stomach was then returned to the peritoneal cavity, and the abdominal wall and skin were closed. 3 d later, all animals implanted with intact tumor tissues received i.v. injection of PBS (group3, n=12) or P-selectin antibody (Suzhou Medical College; 100 μg/injectiom; group4, n=12) twice weekly for 3 weeks. Animals in normal group received i.v. injections of PBS (group1, n=12) or P-selectin antibody (100 μg/injection; group2, n=12).

Sample collection and pathological examination

Two mice in each group were sacrificed randomly on weeks 1, 2,and 4. The remaining animals were sacrificed at 6th week and the tumors growing on the stomach wall were removed and examined histologically. Tissues from all organs were examined for metastasis after careful macroscopic examination. The spleen of mice was harvested for detection of immune function.

Lymphocyte transformation test

Using ^{3}H TdR infiltration method, T cell transformation function was detected with Con A (5 μg/ml, Sigma), B cell function with LPS (50 μg/ml, Sigma). The spleen was made

into suspension and mononuclear cells were acquired with lymphocyte-separating fluid. The cell suspension was adjusted to 2×10^6/ml with RPMI 1640 liquid containing 10 % calf serum (GIBCO). Then 200 μl of the cell suspension was put into each well in 96-well plates. One plate was for Con A group, and another plate for LPS group. There were negative control group and experimental group for Con A or LPS group respectively, and each test had 5 repetitive wells and was cultivated under 5 % CO_2 at 37 ℃. The cell suspension in Con A group was cultivated for 3 d, while it was done for 5 d in Con A group. All wells were added up ^{3}H TdR 16 h before the end of cultivation. They were collected in filtration paper, and given 0.5 ml of scintillating liquid to detect cpm value with β-scintillator, which was presented with SI. SI amounts to cpm of experimental group/cpm of empty group.

NK activity

NK activity was detected by the 4 h LDH release method. NK cell activity (%)=

$$\frac{\text{Experimental group' s OD-Natural release group' s OD}}{\text{Max release group' s OD-Natural release group' s OD}}$$

Statistical methods

Comparisons among groups were performed by the student' s test and χ^2 test. A value of $P<0.05$ was considered significant.

RESULTS

Effects of P-selectin antibody on metastasis

All animals in each group were sacrificed randomly at the 1st, 2nd, 4th week and 6th week. Tumors grew in the implanted site. Under microscopy, the tumors demonstrated lower differentiated adenocarcinoma invadeing mucosal layer, submucosal layer and muscle layer. Tumor metastasis was observed most frequently in the regional lymph nodes and liver. It could be seen in lung, spleen and other organs.

In tumor group (Group 3), no metastasis was found at the 1st and 2nd week, but metastasis was found in 1 of 2 cases at the 4th week, in 5 of 6 cases at 6th week. However, in P-selectin antibody treated tumor group (Group 4), tumor metastasis was remarkably inhibited. No metastasis was found at 1st, 2nd, and 4th week, while it was found only in 1 of 6 mice at 6th week.

Table 1 Immune functions of mice in various groups ($\bar{x}\pm s$)

Group	Week 1	Week 2	Week 4	Week 6
T cell transformation indices (SI)				
Group 1	1.95±0.16	2.19±0.18	2.28±0.19	2.35±0.20
Group 2	2.10±0.19	2.13±0.21	2.42±0.23	2.27±0.22
Group 3	1.83±0.17	1.77±0.18	1.58±0.15	1.01±0.10[d]
Group 4	1.92±0.19	1.82±0.16	1.63±0.17	1.43±0.13[a]
B cell transformation indices (SI)				
Group 1	2.57±0.22	2.35±0.23	2.76±0.24	2.87±0.26
Group 2	2.49±0.24	2.33±0.26	2.82±0.28	2.59±0.29
Group 3	2.54±0.23	2.48±0.24	2.20±0.22	1.49±0.12[d]
Group 4	2.60±0.22	2.51±0.21	2.43±0.26	2.11±0.20[a]
NK activity (90)				
Group 1	8.66±0.78	11.21±0.99	14.22±1.22[b]	18.62±1.14[c]
Group 2	8.96±0.91	11.39±1.12	13.75±1.03	17.99±1.36
Group 3	8.12±0.81	7.63±0.74	6.75±0.62	4.17±0.52[d]
Group 4	8.00±0.73	7.61±0.83	6.97±0.62	6.24±0.63[a]

[a]$P<0.05$ *vs* group3; [b]$P<0.05$, [c]$P<0.01$ *vs* week1; [d]$P<0.05$ *vs* week4.

Effect of P-selectin antibody on immune function

The NK activity of normal mice increased over time, but no significant difference of the immune functions of T cell and B cell was found in all the groups. Over time, the immune functions (T, B cell function, NK activity) of SCID mice orthotopically implanted with tumor tissue were significantly lower in the 6th week than those in the 4th week, but the change was attenuated by P-selectin antibody ($P<0.05$) (Table 1).

DISCUSSION

Research on cell adhesion molecules and metastasis has aroused a lot of attention recently[34-50]. P-selectin (GMP140, CD62, or PADGEM) is located in alpha granules of platelets and Weibel-Palade bodies of endothelial cells. Once platelet or endothelial cell is activated by mediators such as thrombin, alpha granule and weibel-palade body membranes fuse rapidly with the plasma membrane, leading to the expression of P-selectin on the cell surface[29]. P-selectin contributes to interaction of tumor cell to cell adhesion by mediating endothelial cells and platelets with tumor cells.

We have immunohistochemically studied P-selectin expression in 60 cases of human gastric carcinomas. The study showed that P-selectin was expressed in human gastric cancer, which was detected not only on intratumoral endothelium but also on cancer cells. P-selectin expression was found higher in patients with lymph node metastasis than those without metastasis. The survival time and five-year survival rate were lower in P-selectin positive cases than those in negative cases[30-32]. Recently, our study indicated that P-selectin mRNA expression is related to tumor metastasis, and the metastasis may be inhibited by the monoclonal antibody. This finding is in accordance with Stone' s results[22]. These results suggest that selectins play an important role in metastasis by mediating the interaction of endothelial cells and platelets with cancer cells, and the metastasis can be inhibited by the monoclonal antibody.

Recently, study on anti-adhesion therapy with adhesion molecule monoclonal antibodies exhibits good clinical prospective[51-53]. However, there existed some problems. Antibody against intercellular adhesion molecule-1 (ICAM-1) might block inflammation while it inhibits the immune function of the body so that it increases the risk for infection[54,55]. The present study investigated the effects of P-selectin monoclonal antibody on tumor metastasis and immune function of SCID mice orthotopically implanted with gastric cancer tissue to lay foundation for clinical application of anti-adhesion therapy. The result indicated that P-selectin antibody could inhibit the metastasis. This is consistent with our previous study.

Results obtained in this study showed that NK activity of normal mice got higher with the passage of time. It was higher at 6th week than between 1st week and 4th week. But there had no significant change in both T cell and B cell function. The experiment suggested that mice used for metastasis models should not be too old, otherwise the metastasis rate of the mice implanted with tumor would be low. Our experiment discovered that the immune functions (T, B cell function, NK activity) of the tumor group were significantly lower in the 6th week than in the 4th week, but the change was remarkably attenuated by P-selectin antibody. No difference of the immune functions between the two groups at 1st, 2nd, 4th week was found. This result suggested that the immune functions of mice was suppressed with the development of tumor metastasis. On the other hand, the metastatic rates of tumor group were 50 % (1/2) at 4th week and 83.3 % (5/6) at 6th week, whereas it was 16.7 % (1/6) at 6th week in P-selectin antibody treated tumor group. Therefore, the improved immune function in the

antibody treated tumor group might result from the inhibition of metastasis by the P-selectin monoclonal antibody. Sharar discovered that P-selectin could not inhibit functions of neutrophils. The results suggest that P-selectin monoclonal antibody could inhibit the metastatic tendency with no harmful effect on host immune function.

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