Detection and Clinical Significance of Circulating Carcinoma Cells in Peripheral Blood of Oophoroma Patients

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Abstract **Objective** To detect the circulating carcinoma cells in peripheral blood of patients with ovarian carcinoma by the immunomagnetic beads (IMB) , and evaluate the sensitivity of the technique. **Methods** Ovarian carcinoma cell lines OC-3-VGH and OVCA433 cultured in vitro were incubated with monoclonal antibody(anti-CA-125-MAb) and normal peripheral blood of human, then reacted with immunomagnetic beads (IMB) and separated by a magnetic separator. **Results** The optimal rosette formatting rate is 90% when the concentration of anti-CA-125-MAb reaches 20μg/ml and IMB: OC-3-VGH ratio is 15:1. Carcinoma cells can be detected using IMB technique when the ratio of PBMC: OC-3-VGH is 2×10^6:1 and the concentration of OC-3-VGH in blood is 10/ml. **Conclusion** Immunomagnetic beads technique is a high effective, rapid, sensitive and specific method which can be used to detect and separate carcinoma cells in circulation of the patients with ovarian cancer in order to improve the diagnostic rate in early stage of ovarian cancer.

Key words Immunomagnetic beads (IMB); Ovarian cancer; Anti-CA-125-MAb

Oophoroma is one of the popular carcinomas of women in germen, which is the third place next to cervices and uterus cancers in rates of carcinomas. In the forepart of oophoroma, there are metastasis in celiac and micrometastasis. Micrometastasis is the tiny carcinoma tissue which the carcinoma cells proliferate and live in lymph system, peripheral blood, medulla, liver and lung organs during the carcinoma cells developing. The method how to detect the rare carcinoma cells in the blood of patients with malignancy tumor is unexpected. Recently, more and more evidences show that the searches of micrometastases in the lymph system and medulla would directly affect patients´ prognostic, and it is an isolated factor for recurrence and survival, and is the base and the precondition for metastasis and recur.[1~3]

How to detect the carcinoma cells of micrometastasis from peripheral blood and medulla is the attentively problems in clinical diagnose and therapy. This study was designed to detect the circulating carcinoma cells in mimesis of peripheral blood of patients with oophoroma and investigate the sensitive of immunomagnetic beads (IMB) methods.

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MATERIALS AND METHODS

Materials

Human oophoroma OC-3-VGH cell line was provided by Department of Cells and Biology, China Medical University. Human oophoroma OVCA433 cell line was provided by Third Clinical Hospital, Beijing Medical University. Mouse -anti -`human CA-125-monoclonal antibody was purchased from Fujian Maixin Biotechnics Developing Co., China. Immunomagnetic beads (IMB) coating with goat-anti-mouse IgG (type M-450) was purchased from Dynal Company, Norway.

Methods

The culture of ovarian carcinoma cells

Oophoroma OC-3-VGH cells and OVCA433

cells were cultured by RPMI1640 containing 10% fetal calf serum, which were dispensed into 1×10^7/ml suspension respectively. Cell activity was detected and CA−125 immunohistochemical examination was carried out.(Fig. 4 and 5)

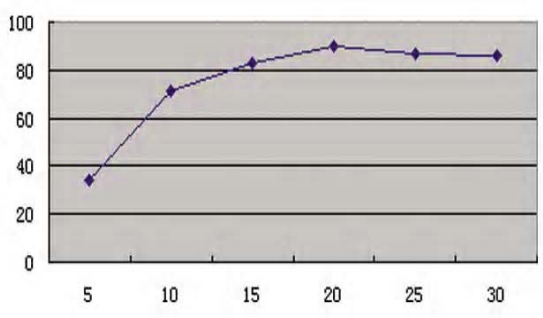

Fig.1: Relationship between the rosette formation ratios of OC−3−VGH cells and concentrations of anti−CA−125 monoclonal antibody.

Note: Abscissa was the concentrations of monoclonal antibody (μg/ml) and y−axis was the ratios of formation of rosette(%).

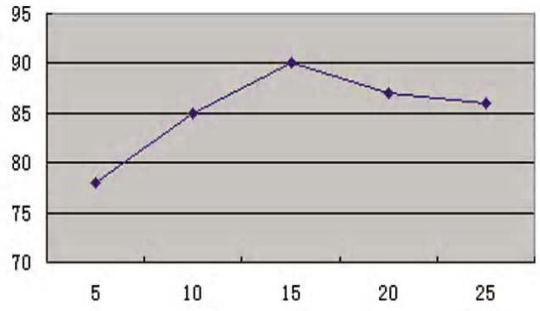

Fig.2: Relationship between the rosette formation ratios of OVCA433 cells and concentrations of anti−CA−125 monoclonal antibody

Note: Abscissa was the concentrations of monoclonal antibody (μg/ml) and y−axis was the ratios of formation of rosette(%).

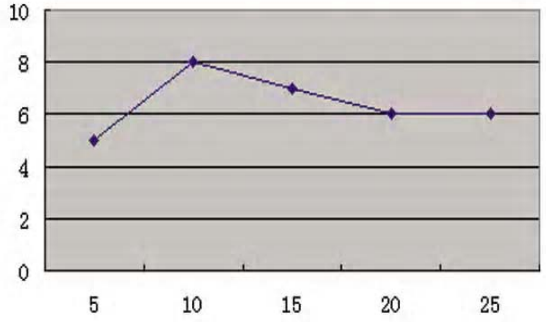

Fig.3: the relationship between rosette formation ratios of OC−3−VGH cells and the rations of cells with IMB.

Note: Abscissa was the ratios of IMB with OC−3−VGH cells. Y−axis was the formation ratios of rosette (%).

Detection of specificity of IMB

Certain amount of CA−125 monoclonal antibody was added with sufficient effect at 4℃ for 90 min. Cells were washed out by PBS solution and recovered to the original volume. IMB M−450 washed by PBS was added according to the ratio of cells to IMB of 1:5~1:25, and slowly shook at room temperature for 60 min. The cells combined with 3 and over 3 IMB were taken as positive cell (the formation of rosette), and judge the combination of different cells with IMB under different reaction conditions.

Immunodetection

(1) PBMC suspension was prepared.

(2) The logarithmic growth OC−3−VGH cells were added into PBMC suspension proportionally, the proportions were 10^4:1, 5×10^4:1, 10^5:1, 2×10^5:1, 5×10^5:1, 10^6:1, respectively.

(3) Anti−CA−125 monoclonal antibody and IMB M−450 were added into the cell suspension, which was placed under magnetic separator to observe the carcinoma cells.

Cell isolation

(1) Anticoagulant blood was taken from healthy people. OC−3−VGH cell in the number of 1000, 500, 200, 100, 50, 20, 10, 1 was added in each milliliter blood, respectively.

(2) Anti−CA−125 monoclonal antibody was added and inoculated at 4℃ for 90 min. The excessive antibody was washed out, and IMB M−450 added, effected at room temperature for 60 min to magnetic separation. Carcinoma cells were observed.

Statistics

χ^2 test was adopted for analysis of statistical difference, and $P<0.05$ was taken as significant difference.

RESULTS

Detection of specificity of IMB

There were more OC−3−VGH cells combined

IMB informing rosette to be seen under the optical microscope, but it was rare to OVCA433 cells in same conditions. (Fig. 8) After mixed with anti−CA−125 monoclonal antibody, the most combining ratio of OC−3−VGH cells, OVCA433 cells and PBMC with IMB (M−450) were 90%, 8% and 0% respectively. The 125 immunohistochemical were 70%, 55% and 0% respectively. Analysis of statistics showed that the difference between OC−3−VGH cell and other two cells were significance (P<0.001).

When concentration of cells, ratio of IMB and cells were fixedness, changing the concentration of antibody, the different ratios of rosette formation would be gotten (Fig.1 and 2). Added different concentrations of antibody from 0 to 20μg/mL (2μg/10^6 cells), the rosette formation ratio of OC−3−VGH cells was up to 90%. If increasing the concentrations of antibody further, the rosette formation ratio didn´t follow and even decreased. When concentrations of antibody were 10μg/mL (1μg/10^6 cells), the rosette formation ratio of OV-

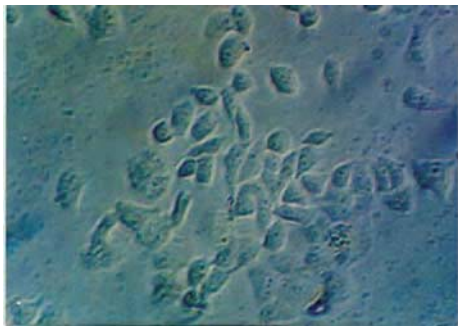

Fig.4 The cells of OC−3−VGH (200×)

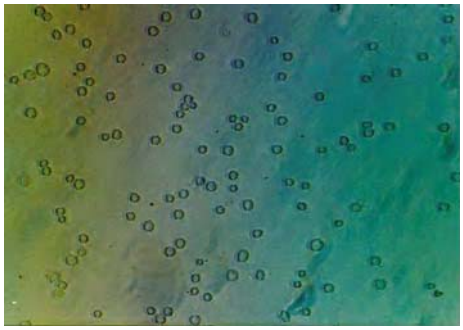

Fig.5 The cells of OVCA433 suspend in the water. (200×)

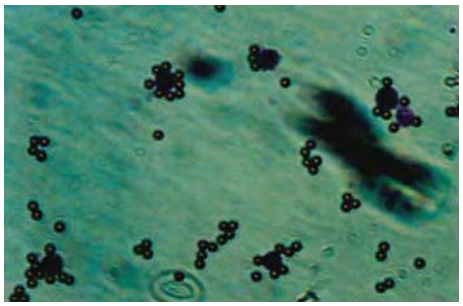

Fig.6 The cells OC−3−VGH covered with mono−clone body CA−125 combined with IMB. (IMB: cells=5:1) (400 ×)

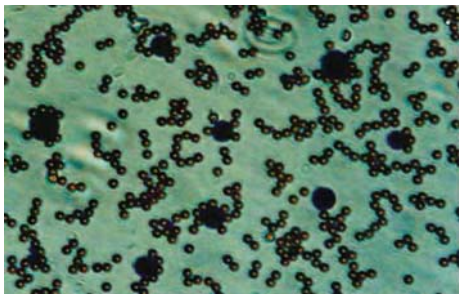

Fig.7 The cells OC−3−VGH covered with mono−clone body CA−125 combined with IMB. (IMB: cells =15:1) (400×)

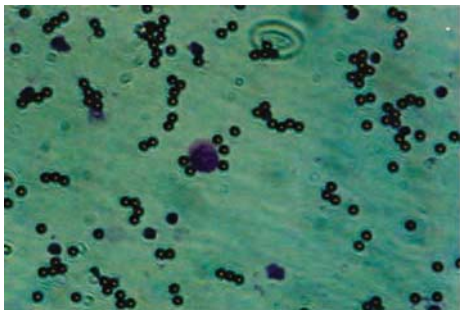

Fig.8 The cells OVCA433 covered with mono−clone body CA−125 combined with IMB. (IMB: cells= 5:1) (200×)

CA433 cells was 8%. The combination ratio keep stability even increased concentrations of antibody.

When the concentration of OC−3−VGH cells was invariable and antibody was 20μg/mL, if the ratio of cells and IMB was changed, the rosette formation ratio was changed also (Fig.3). When ratios of cells and IMB changed from 5:1 to 15:1, rosette formation ratio was followed to the most, and when it increases continued, the ratio of rosette was no changed no more.(Fig.6 and 7)

Immunodetection

10^7 PBMC were obtained from 10 milliliter of anticoagulant blood in health people and then added OC−3−VGH cells in number of 1000, 200, 100, 50, 20, 10 respectively. The concentration ratios of PBMC to OC−3−VGH cells were 10^4:1, 5×10^4:1, 10^5:1, 2×10^5:1, 5×10^5:1, 10^6:1, 2×10^6:1, 5×10^6:1, 10^7:1 respectively. Five tests were done every concentration. The test result showed that when concentration was 2×10^6:1, OC−3−VGH cells were detected three times and no detected in 5×10^6:1, 10^7:1. Oophoroma cells could be detected in other concentrations.

Cell isolation

Added OC−3−VGH cells in number of 1000, 500, 200, 100, 50, 20, 10, 1 to 1 milliliter fresh anticoagulant blood respectively. Five tests were done every concentration. OC−3−VGH cells were not detected in one−cell group and got three times in ten−cell groups. In other groups carcinoma cells could be detected which combined IMB forming rosettes.

DISCUSSION

Oophoroma was the first one for the death of patients in gynaecological carcinomas, it was no symptoms and no exactly detecting methods in early period of disease.

IMB technique is a kind of new immunological techniques studied at home and abroad in recent years. IMB are ball−like magnetic microns coating with monoclonal antibody, which can combine with specific target substances and so have magnetic responses. Someone abroad reported that the proliferation of $CD34^+$ cells isolated from peripheral bloods of 19 patients with stage Ⅳ mammary cancer with IMB technique was made and at last the proliferated cells were infused into the patients´ bodies again to overcome the side effects brought by large doses of chemotherapy for many times [4]. Scholars at home have reported that modified enrichment of IMB and immunofluorescence cytochemical tests were adopted to detect the circulatory cancer cells in peripheral blood of patients with mammary cancer[5]. Besides, IMB technique has been applied in isolation or clearance of specific component of cells in all kinds of medical and scientific researches.

Data showed that, as far as the only item of indexes concerned, CA−125 was one of the most valuable carcinoma markers in clinical diagnosis of oophoroma at present [6]. In the early check−up, serum CA−125 was taken as an economical and convenient early detection means[9].

IMB technique offered a new research method to detect micrometastasis of oophoroma. The correlative questions of this test and the feasibility in clinic application of IBM was analyzed as follows:

1. The test adopted new type IMB (Dynal−450) coated with goat−anti−mouse IgG, the ball−like configuration of IBM dismissed the unspecific combination with erose microne and so they could combine with anti− CA−125 monoclonal antibody tightly on the cells´ surface. When IMB were put in the magnetic field, super−paramagnetism could display magnetic response, wipe off the magnetism and spread around the beads when moving them. Compared with the existing separateness techniques, the method could not only obtain high pure cells but not influence the cells´ activity. For example, in the test, the cells´ activities had no obvious variety in the immunocytochemistry detection and separation the OC−3−VGH cell. Before separation was 96% and after that was 94%. The method has more merits, for instance, simply, speediness, no costly equipments and easy to separate and pure in asepsis. So it should be popularized in clinic.

2. The method was indirect technique and the result of two oophoroma cells immunodetection showed that the combination ratios to IMB (M−450) were different

between OC−3−VGH cell line (90%) and OVCA433 cell line(8%). The reasons may be as follows:

(1) The CA−125 antibody had three types in oophoroma cells based the immunocytochemistry pigmentations of oophoroma cells, for example, membrane type, plasma type and interstice type. In two cell lines, the CA−125 antibody was settled in cell membrane of OC−3−VGH cells, so it was membrane type. While OVCA433 cells was in plasma and it was plasma type. Antibody only could combine with antigen on the membrane if cell kept full. So anti−CA−125 monoclonal antibodies may react adequately with antigens on the surface of the OC−3−VGH cell membranes and obtain the approving combination ratio. While the antibodies of CA −125 were rare on the cell membranes of OVCA433 cells, it would not increase combination ratio even if added the anti−CA−125 monoclonal antibodies.

(2) There were innumerability antigens on the molecule of CA −125 and the structures of these antigens were very discrepancy in differnt human cancers and different differentiation of carcinoma cells. Owing to the complication of CA−125 antibodies´ structure, multiplicity of antigens and correlative antigens´ existence, the CA−125 antibody has a big and complex family. So the matching on combining with same antibody structure would be some discrepancy and influence the combination ratio of antigen and antibody.

3.The influence factors of formation ratio of rosette.

(1) The type of cell. The more differences of rosette formation ratio would be existed to different cell lines. The ratio of OC −3 −VGH cell line combining with IMB even could be 90%. But in the same condition, the ratio of OVCA433 cell line only was no more then 8%. The difference in the result owing to the different cell lines would influence the use of this method in clinic for a certain extent.

(2) The type of monoclonal−body. It was significant to choice efficient monoclonal−body for obtaining the farthest effects of IMB. The monoclonal−body of this test was mouse antibody, IgG2a, and the IMB M −450 adapted to the types of monoclonal−body IgG1, IgG2a and IgG2b. So the combinations between them were better. Thus, the correct monoclonal−body choice was most important to obtain the perfect results.

(3) The concentration of monoclonal−body. When other conditions were made certain, the combinations of antigen and antibody would tend to saturation following increase of the concentration of antibody. Overabundant antibody would join each other in structure and effect the combinations with IMB and consequently the rosette formation ratio was low in certain extent.

(4) The proportional relationship of cell and IMB. If the amount of IMB was more, it wouldn´t add up the total ratio of rosette formation and only increase the number of combination of IMB in positive cells and decrease the response time of reaching top combination ratio.

(5) Physical conditions. Put IMB and cells which combined with monoclonal−body at room temperature and shake slowly to blend appropriately. The rosette formation ratio was more than which inoculated in 4℃. The ratio of combination was more in 60 min then in 30 min. Thus, temperature and time were either importance to combine of cells and IMB. Insurance enough action between cells and IMB could increase the combination ratio.

4.The result of separating cells from blood showed that ten OC −3 −VGH cells could be detected from 1 milliliter blood. It was the theoretic and practical technical bases for separated carcinoma cells from thorax, abdomen, pericardiac cavity and so on. The analysis sa follows:

(1) Blood contains serum, red cells, white cells, platelets and manifold antigens. It would effect the special combination of monoclonal−body with aim antigen.

(2) Cells were washed reiteratively when separated from blood and so the antigen character of carcinoma cells was destroyed. This effected combination between antibody with cells and IMB with cells. So the ratio of separation was low.

(3) The solid components in suspension, such as plentiful red cells, white cells, platelets, would disturb the combination in antibody, IMB and carcinoma cells.

Hereinbefore, it showed that the method in the test utilized high expression of CA−125 on oophoroma cells and adopted IMB technique to increase the ratio of detecting carcinoma cells from blood. The method only needed antibody and magnet. Its merit was simpleness, trustiness and speediness to operate. It would increase

the effect of detected cells if could find better conditions and operating methods and so it could be used in clinic.

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