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Immuno-Histological Characterization of OVS_1 and OVS_2 Monoclonal Antibodies Recognizing Human Ovarian Mucinous Cystadenocarcinoma

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SUMMARY **OVS_1 and OVS_2 monoclonal antibodies (MAbs) were established by fusing murine myeloma cell line NS1/1-Ag4-1 with mouse spleen cells immunized with fresh human ovarian mucinous-cystadenocarcinoma tissue. The selection of the MAbs was assayed by an immuno-histological (streptavidin-biotin) staining of the specific antigen antibody reaction localized on frozen sections of the same tumor. Other paraffin sections and established cell lines were also screened by immuno-histological staining in order to characterize the specificity and sensitivity of these two MAbs. OVS_1 MAb showed 96% specificity and 67% sensitivity to mucinous cystadenocarcinoma with no cross reactions to normal tissue, benign tissue, other cancers, or any established cell lines. OVS_2 MAb revealed only 8% specificity but 78% sensitivity to mucinous cystadenocarcinoma, however, a cross reaction to some normal and benign tissues or other cancers was shown. The data suggested that OVS_1 and OVS_2 MAbs could be used in combination to detect ovarian mucinous cystadenocarcinoma.**

Ovarian cancer is one of the most lethal[1] gynecological malignancies because the tumor is often severely advanced by the time of clinical diagnosis. Approximately two-thirds of cases go to see gynecologists at late stage since early stage of the disease is usually asymptomatic; early detection by physical examination, and other conventional investigations such as X-ray or ultrasound have not been helpful. The overall 5 year survival rate of ovarian cancer[1] varies between 42-70% for early stages, and 4-13% for advanced stages. Many attempts have been made to develop reliable tests for the detection of specific tumor markers that can be used for screening, and for monitoring the response of treatment or detecting early recurrence of the tumor.[2-18]

CA 125[9,19-22], the most widely used for the diagnosis of epithelial ovarian cancers especially non-mucinous type, has been reported (similar to CEA and CA 19-9) to be nearly 50% positivity for mucinous cystadenocarcinoma. The new ovarian tumor markers, STN[23], CA 546[24] and CA 72-4[25] could give a positive rate for mucinous cancer as high as 63% and show some cross positivity with benign tumors and inflammatory tissues. Since mucinous and serous cystadenocarcinoma along with endometrial cancer are the most frequently encountered ovarian cancers, particularly in Thailand,[26,27] the development of new monoclonal antibodies recognizing antigens with greater sensitivity and specificity for mucinous ovarian cancers would be clinically useful.

We have established new monoclonal antibodies (MAbs) that could recognize more specifically ovarian mucinous cystadenocarcinoma. Immuno-histological staining of frozen tissue sections were used to identify the specific reactions between the tumor antigens and the established MAbs.

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MATERIALS AND METHODS

The NS1/1-Ag4-1 murine myeloma cells [28] were fused with splenocytes from BALB/c mouse immunized with human ovarian mucinous cystadenocarcinoma tissue. The cancer tissue was minced immediately after surgical removal and adjusted to 10^5 viable cells/ml then aliquoted and kept frozen in liquid nitrogen to be ready for injection. The protocol was as follows: the mouse was given two intraperitoneal injections with 500 µl of minced cancer tissue, followed by one intravenous injection with 10 µl of the cell suspension on each of days 47, 20 and 4 before sacrifice, respectively. Hybridoma-supernatants containing immunoglobulins were screened by immuno-histological staining of frozen tissue sections prepared from the same tumor that was used to immunize the mouse. The histostain-SP kit (Zymed Laboratories Inc, San Francisco, USA.), based on a biotinylated streptavidin-peroxidase method was used, showing positive recognition as a pink staining on the tumor but no color staining on stroma or normal tissues of the same patient (Fig. 1). Once hybridomas in each well showed specific staining, cells were then immediately cloned by limiting dilution and soft agar cloning techniques to obtain monoclonality. The selected hybridoma cell lines after cloning were propagated in mouse ascitic fluid in order to collect large amounts of antibodies. Purification of MAbs from ascites was performed by ammonium sulfate precipitation followed by protein-A-Sepharose chromatography (Pharmacia Technology Co, Uppsala, Sweden). [28]

Established MAbs were then screened for immunoglobulin subclasses by fraction chromatography and dot blotting techniques. [28] The specificity, sensitivity, positive and negative predictive values for mucinous ovarian cancer were calculated [29] from the results of the immunohistological staining by the MAbs in normal or various human cancer tissues and cell lines.

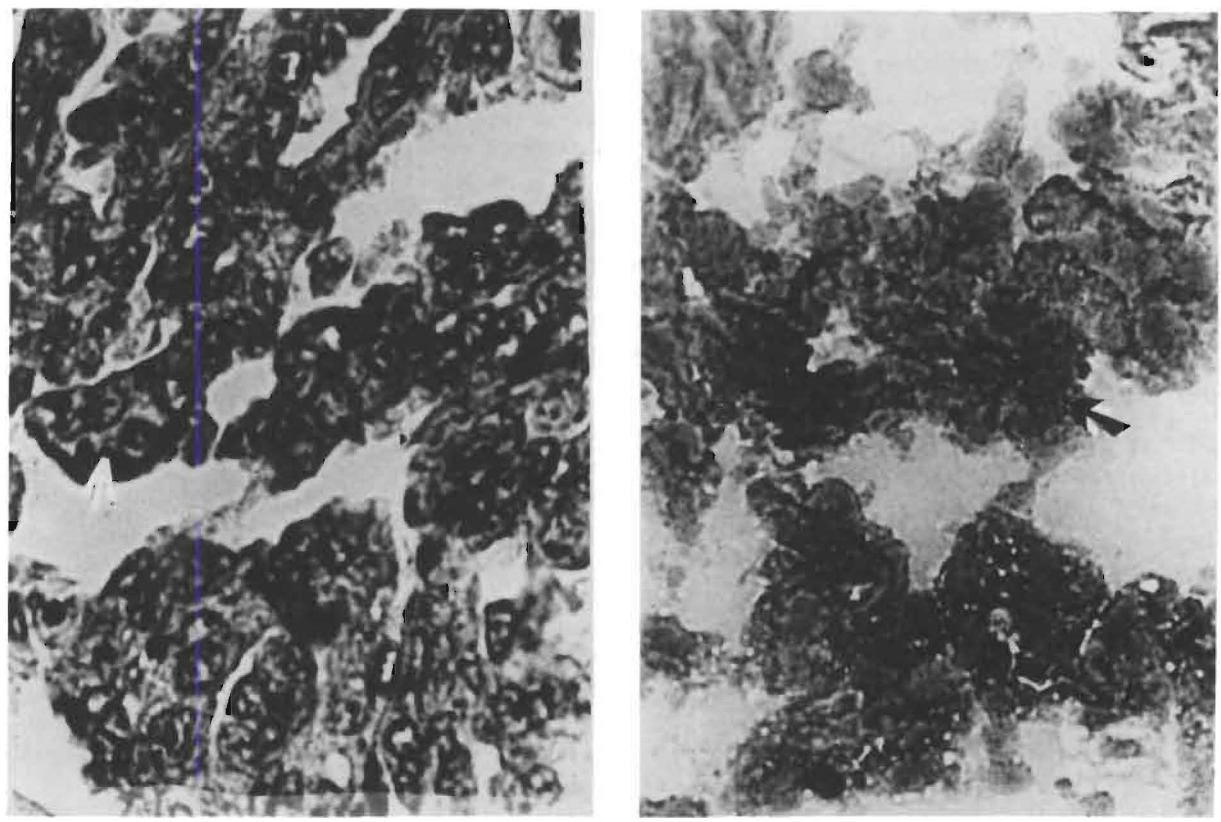

Fig. 1 Negative and positive immuno–histological (streptavidin–biotin) taining of normal (left) and cancer (right) fissue sections using OVS_1 and OVS_2 MAbs. The cancer area showed a positive pink color staining (right), while stroma and normal tissue gave no color (left).

RESULTS

The splenocytes of the immunized mouse, with its serum titer over 1:16,000 and showing positive immuno-histological staining on frozen ovarian cancer sections, were used for hybridoma production. After the fusion, hybridomas were obtained from 336 out of 480 wells (70%). Among these, 60 from 336 wells (12.5%) were IgG secreting clones. Furthermore, only MAbs derived from 11 of 60 clones (18.3%) gave a strong reactivity as demonstrated by immuno-histological staining on tissue sections. Although seven of 11 clones revealed no reaction on stromal tissue, after the second cloning, only 2 of the 7 clones that produced OVS_1 and OVS_2 MAbs gave specific recognition on the tumor tissue and they were propagated as permanent hybrid cell lines (Fig. 2). The staining of OVS_1 MAb was evenly distributed in the cells while OVS_2 MAb staining was observed on both luminal and basal part of cells. The OVS_1 and OVS_2 MAbs produced by the 2 cell lines in mouse ascites, after purification by ammonium sulfate and protein-A chromatography, were identified to be IgG_1 isotype.

The reactivities of OVS_1 and OVS_2 MAbs on normal and benign tumor tissues, mucin producing non-ovarian cancer, ovarian cancer and some established cell lines are shown in Tables 1-3. The OVS_1 MAb showed a highly specific staining reaction with ovarian mucinous cystadenocarcinoma (12/18); slight reaction with serous cystadenocarcinoma (1/12); but no reactivity to other types of ovarian cancer (0/12), other mucin producing non-ovarian cancer (0/11) or established cell lines (0/21) was observed. On the contrary, OVS_2, MAb recognized antigens in almost all ovarian cancers, regardless of types (36/42), as well as some mucin-producing non-ovarian cancers (7/11), benign conditions (6/8), and normal tissues (5/20). None of the established cell lines (2 normal, and 19 cancer cell lines) were recognized by these MAbs.

The calculated values of specificity, sensitivity, positive and negative predictive values for the staining

Table 1. Immunohistological staining results of OVS_1 and OVS_2 MAbs on normal, benign, non–ovarian cancer, and ovarian cancer tissues. The calculated specificity (spec.), sensitivity (sens.), positive predictive value (PPV) and negative predictive value (NPV) are shown.

Tissue staining	Total cases	Positive staining/total					
		OVS_1	% spec.	% sens.	OVS_2	% spec.	% sens.
1. Normal tissues (ovary, uterus, stomach..)	20	0/20			5/20		
2. Benign condition (myoma,endometriosis..)	7	0/7			6/8		
3. Mucin producing non–ovarian cancer	11	0/11		0	7/11		64
4. All ovarian cancer	42	13/42		31	36/42		86
Mucinous	18	12/18	96	67	14/18	8	78
Serous	12	1/12			11/12		
Other	12	0/12			11/12		
PPV for mucinous ovarian cancef		92%			39%		
NPV for mucinous ovarian cancer		79%			33%		

spec : % of the non–mucinous ovarian cancer tissues with negative staining
sens : % of the mucinous ovarian cancer tissues with positive staining.
PPV : % of the ovarian cancer tissues with positive staining, showing the possibility to be mucinous type.
NPV : % of the ovarian cancer tissues with negative staining, showing the unlikeliness to be mucinous type.

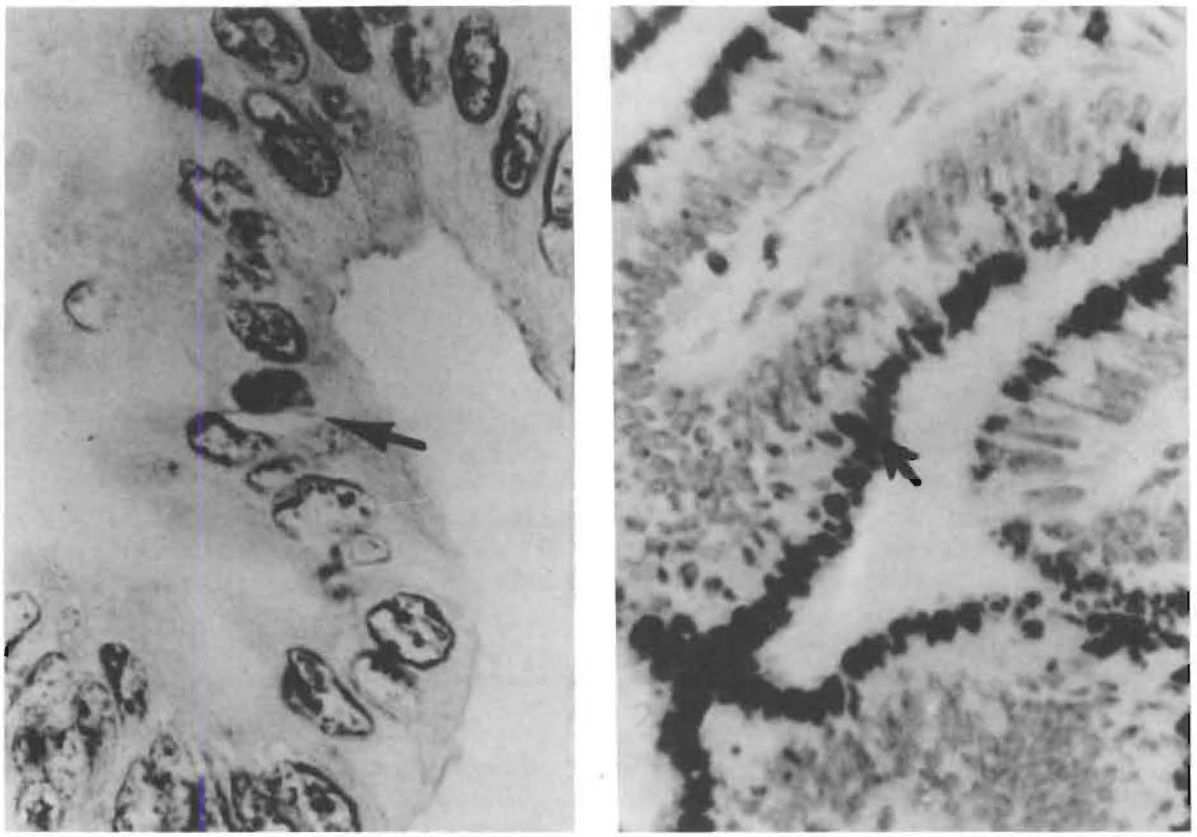

Fig. 2 The comparison of the localization of OVS_1 (A) and OVS_2 (B) MAb on the paraffin embedded sections of mucinous cystadenocarcinoma, (A), the localization of OVS_1 MAb was generally distributed in the cell, (B), the localization of OVS_2 MAb was diffuse and more intense on both luminal and basal parts of the cell.

Table 2. The immuno–histological staining results of OVS_1 and OVS_2 MAbs on mucin producing normal tissues

Organ	No. of cases	Positive staining/total	
		OVS_1	OVS_2
Normal tissue			
1. Endometrium	3	0/3	0/3
2. Cervix	6	0/6	3/6
3. Ovary	5	0/5	0/5
4. Stomach	2	0/2	0/2
5. Colon	2	0/2	0/2
6. Uterine tube	2	0/2	2/2
Total	20	0/20	5/20

Table 3. The immuno–histological staining results of OVS_1 and OVS_2 MAbs on mucin producing non–ovarian cancer, and benign non–ovarian tissues.

Organ	No. of cases	Positive staining/Total	
		OVS_1	OVS_2
Malignant tissue			
1. CA Endometrium	2	0/2	2/2
2. CA Stomach	2	0/2	2/2
3. CA Colon	2	0/2	2/2
4. Cholangiocar–cinoma	2	0/2	1/2
5. CA breast			
: Invasive ductal CA brease	2	0/2	0/2
: Mucinous CA breast	1	0/1	0/1
Total non–ovarian cancer tissue	11	0/11	7/11
Benign tissue			
1. Myoma	2	0/2	1/2
2. Serous adenoma	1	0/1	0/1
3. Mucinous adenoma	4	0/4	4/4
4. Endometriosis	1	0/1	1/1
Total benign tissue	8	0/8	6/8

results of OVS_1 MAb were 96%, 67%, 92% and 79% respectively, while the corresponding values for OVS_2 MAb were 8%, 78%, 39% and 33%, respectively.

DISCUSSION

The establishment of OVS_1 and OVS_2 MAbs by using fresh ovarian cancer tissue from a patient as the immunogen, was different from other reports that mostly used established cell lines.[9,19-25] We immunized mice with fresh frozen tissues to obtain sensitized cells for producing MAbs that would recognize well-preserved tumor antigens. However, heterogeneity of the immunizing antigens in the tumor tissues could not be avoided. It required a good screening technique to obtain the specific MAbs. Since we could not establish or keep tumor cell-lines growing in media after surgical removal, we chose the immuno-histological technique for our screening method. The staining reaction showed specificity and sensitivity of the OVS_1 MAb on paraffin section, comparable with the results from ELISA.[29]

The staining patterns of OVS_1 and OVS_2 MAbs on frozen tumor sections were somehow different. Although both MAbs showed diffuse antigen recognition all over the cell, more marked staining in the basal and luminal part of cells was seen with OVS_2 MAb (Fig. 2). This suggested that the two MAbs recognized different antigens. The results from immuno-histological staining on other paraffin sections of normal and cancer patients (Table 1), also confirmed the different epitopic recognition of OVS_1 and OVS_2 MAbs. In this study, OVS_1 and OVS_2 MAbs could detect particular antigens as shown by the positive staining of the paraffin cancer tissue section after deparaffinization. We conclude that the specific tumors antigens recognized by OVS_1 and OVS_2 MAbs were well preserved in paraffin sections so that we could screen the staining reactivity with these sections instead of frozen tissue sections. OVS_1 MAb showed a high specificity of 94%, with 71% sensitivity in recognition of mucinous cystadenocarcinoma antigen, and no reaction to normal or benign tissues or other non-ovarian cancer cells. The OVS_2 MAb showed poor specificity but very high sensitivity to all ovarian cancer tissues, plus some false positive in normal tissue and some cross-reaction with benign conditions and other non-ovarian cancers.

Thus, despite the likely significant false negative rate with OVS_1, MAb, its specificity suggests real value in diagnostic potential. On the other hand, OVS_2 MAb has only limited value as a specific diagnostic that may possibly be useful for broad screening purpose.

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