ULTRASTRUCTURAL LOCALIZATION OF CA 19–9 IN OVARIAN CANCER

MORIMASA MATSUTA, TERUO KAGABU AND IWAO NISHIYA

Department of Obstetrics and Gynecology, Iwate Medical University, School of Medicine, 19-1, Uchimaru, Morioka 020

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Four cases of ovarian cancer (mucinous cystadenocarcinoma, serous cystadenocarcinoma, endometrioid carcinoma and metastatic cancer of the colon) which revealed diffuse localization of carbohydrate antigen 19–9 (CA 19–9) in their tumor tissues were subjected to immunoelectron microscopy. In all cases CA 19–9 was present in plasma membranes, microvilli and glycocalyx in mucinous and metastatic carcinoma, indicating that the antigenic determinant for CA 19–9 is sialylated Lewis A. Reaction products were seldom seen in perinuclear spaces and endoplasmic reticula. Many pictures revealed positive vesicles apparently released from the Golgi apparatus; this feature seemed to be characteristic of the findings. Thus, the Golgi apparatus is considered to be the place where immunoreactive CA 19–9 is first produced in cancer cells. Since positive images were found not only in organelle but also on collagen fibers directly under the basement membranes, it seemed that an important factor for CA 19–9 increase in the blood is the expansion of CA 19–9 to stroma.

It was strongly suggested that CA 19–9 was useful as a tumor marker for ovarian cancer which originated in common epithelial cells.

Many tumor markers have been developed to detect ovarian neoplasms because of various histological pictures. Some of these markers have been used for supplementary diagnosis, monitoring the effects of treatment and checking for recurrence.

Carbohydrate antigen 19–9 (CA 19–9) (5) is known as a useful tumor marker for cancer of the pancreas and colon (5, 6, 7). It has also been reported that approximately 30% of ovarian cancer patients showed an increase of CA 19–9 value in the blood (1). Immunohistochemical studies at light microscopic levels have revealed the localization of CA 19–9 in cases of pancreatic and ovarian cancer (7, 11). An observation using immunoelectron microscopy revealed the ultrastructural localization of CA 19–9 in chronic liver disease, indicating that CA 19–9 is useful as a tumor marker in the biliary duct (2). There have been no other reports pertaining to localization of CA 19–9 using electron microscopy.

Ovarian cancer tissues showing diffuse localization of CA 19–9 at light microscopic levels were subsequently examined by immunoelectron microscopy. We also discussed the synthesis, intracellular transportation and secretory mechanism of CA 19–9.
MATERIALS AND METHODS

For the preliminary experiment, ten patients having ovarian cancer, with a certain level of serum CA 19-9 (37 U/ml), were used as subjects. To detect the localization of CA 19-9 in neoplastic tissues at light microscopic levels, the immunoperoxidase method was used and there was no evidence of localization or only partial localization of CA 19-9. The 4 cases of ovarian cancer, revealing diffuse localization of CA 19-9 in tumor tissues, were subjected to immunoelectron microscopy. Outlines of histological types and clinical conditions are summarized in Table 1. Three cases of primary ovarian cancer (mucinous cystadenocarcinoma, serous cystadenocarcinoma, and endometrioid carcinoma) and one case of metastatic ovarian cancer (colonic cancer) are included. The specimens resected at operation were fixed in 10% formalin solution and embedded in paraffin for standard histological and immunohistochemical studies at light microscopic levels. To determine the localization of CA 19-9 under light microscopic observation, the avidin-biotin-peroxidase complex (ABC) method (4) was used as previously described (8). Briefly, the deparaffinized sections were incubated in 0.3% hydrogen peroxide methanol, then washed in 0.01 M phosphate buffered saline (PBS) at pH 7.4. Sections were first incubated with anti-CA 19-9 monoclonal antibody (Cento Core, France) at room temperature for 30 min; second with biotinized anti-mouse IgG serum for 30 min, and finally with avidin-biotin-peroxidase complex (Vector-stain ABC kit, Vector, USA) for one hr. The sections were then stained with diaminobenzidine, and counterstained with hematoxylin.

Immunoelectron microscopic observation: For electron microscopic observation of CA 19-9, the indirect immunoperoxidase method was used (10, 12). Small pieces of tissues were fixed in periodate-lysine-paraformaldehyde solution (PLP) (9) at 4°C for 30 min. The tissues were frozen after a thorough washing in PBS with 20% sucrose for two days. Cryostat sections (4–6 μ) were made, attached to albumin-coated glass slides, incubated with anti-CA 19-9 monoclonal antibody at 4°C for 48 hr, and then subsequently incubated with anti-mouse IgG (goat IgG/Fab') conjugated with horseradish peroxidase (MBL Ltd., Nagoya) at 4°C for 12 hr. After being fixed in 1% glutaraldehyde, the sections were incubated in 0.02% 3,3'-diaminobenzidine 4 HCL (DAB, Wako Pure Chemical Co.) and 10 mM sodium azide (Sigma) in PBS for 30 min and subsequently in the above DAB solution with 0.05% hydrogen peroxide for 3 min. The sections were postfixed in 2% osmic acid in PBS. Following alcohol dehydration, the sections were embedded in Epon 812 by the

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Clinical Stage (FIGO)*</th>
<th>Histological Diagnosis</th>
<th>Serum CA 19-9 U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>—</td>
<td>Metastatic adenocarcinoma (colon, well differentiated)</td>
<td>1,675</td>
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<tr>
<td>2</td>
<td>48</td>
<td>lc</td>
<td>Mucinous cystadenocarcinoma</td>
<td>3,775</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>lb</td>
<td>Serous cystadenocarcinoma</td>
<td>825</td>
</tr>
<tr>
<td>4</td>
<td>63</td>
<td>la</td>
<td>Endometrioid carcinoma</td>
<td>1,100</td>
</tr>
</tbody>
</table>

* International Federation of Gynecology and Obstetrics
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inverted capsule method. The ultra-thin sections were observed by H-700 electron microscope without electron staining.

RESULTS

The localization of CA 19-9 by light and electron microscopy are summarized in Table 2.

1. Light microscopic enzyme antibody method

(a) Metastatic ovarian cancer: The localization of CA 19-9 was observed over the entire intraluminal surface of the glandular formation of tumor cells, also in the intraluminal mucus and stroma. Some tumor cells revealed granular positive images in the cytoplasm (Fig. 1A). In primary colonic cancer tissue, similar findings were obtained. Normal colonic epithelium adjacent to the cancer tissue revealed a strongly positive reaction in the luminal surface, but the intensity of positive images tended to decrease toward the base of the crypt gland.

(b) Mucinous cystadenocarcinoma: Thick zonal positive images were observed on the surfaces of neoplastic cells proliferating toward the cyst. Dot-like positive images were rarely seen in the cytoplasm of cancer cells. Localization of CA 19-9 was also observed in the intraluminal mucus of the glandular formation (Fig. 1B).

(c) Serous cystadenocarcinoma: Localization of CA 19-9 was observed in the apical parts of cells forming glandular structures. Although the localization of CA 19-9 was rarely seen in series over the entire luminal surface, positive images resembling thin lines were obvious in many pictures. Granular positive images were recognized in the cytoplasm of cancer cells. Additionally, weak positive images were also seen in the intraductal substance and stroma (Fig. 1C).

| Table 2. Results of Light and Electron Microscopic Immunostaining for CA 19-9 |
|----------------------------------|----------------|------------------|----------------|----------------|
| Case No. | 1 | 2 | 3 | 4 |
| Materials | Metastatic adenocarcinoma | Mucinous cystadenocarcinoma | Serous cystadenocarcinoma | Endometrioid carcinoma |
| Intraluminal Substance | + Mucus | + Mucus | + | + |
| L.M. Luminar Surface | + | + | + | + |
| Cytoplasm Focal Diffuse | + | + | + | + |
| Glycocalyx | + | + | | |
| Microvilli | + | + | + | + |
| E.M. Membrane Apical Basolateral | + | + | + | + |
| Intracytoplasmic Vesicles | + | + | + | + |
| Golgi Complex | | | | + |
| Endoplasmic Reticulum (+) | (+) | | | |
| Perinuclear Space (+) | (+) | | | |

Fig. 1. Light microscopic localization of CA 19-9
A: Metastatic ovarian cancer  B: Mucinous cystadenocarcinoma
C: Serous cystadenocarcinoma  D: Endometrioid carcinoma
CA 19-9 is present in the luminal surface and cytoplasm. Note the characteristic localization in the stroma (arrows). ABC method. Counterstain with hematoxylin. ×100
Fig. 2. Metastatic ovarian cancer
A, B: Immunoelectron microscopic localization of CA 19-9. Reaction products are seen in plasma membranes, intracytoplasmic vesicles and perinuclear spaces.  A. ×7,500, B. ×9,000
2. Electron microscopic enzyme antibody method

(a) Metastatic ovarian cancer: Reaction products were recognized in perinuclear spaces, endoplasmic reticula, intracytoplasmic vesicles, microvilli, and glycocalyx as well as the intraductal mucus (Figs. 2A, B).

(b) Mucinous cystadenocarcinoma: Reaction products were observed in the glycocalyx, microvilli and intraductal mucus (Fig. 3).

(c) Serous cystadenocarcinoma: CA 19–9 was present in the apical membranes, basolateral membranes, and microvilli as well as in the Golgi apparatus and intracytoplasmic vesicles (Figs. 4A, B).

(d) Endometrioid carcinoma: The localization of CA 19–9 was observed in the microvilli, plasma membranes, intracytoplasmic vesicles directly under the plasma membrane, and the Golgi apparatus. Reaction products were also seen in perinuclear spaces and endoplasmic reticula in a small number of cells. In addition, there was diffuse distribution in the surrounding collagen fibers adjacent to the basement membranes of cancer cells (Figs. 5A, B, C).
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Fig. 4. Serous cystadenocarcinoma
A, B: Immunoelectron microscopic localization of CA 19–9
CA 19–9 is present in the plasma membrane and Golgi apparatus. A, B. ×30,000
DISCUSSION

Since it was assumed that the antigenic determinant for CA 19-9 is sialylated Lewis A (6) and the antigenicity is lost through sialydase digestion (7), a solution used for the purpose of sugar fixation should not be used in immunohistochemical studies of CA 19-9. In the present study we applied PLP fixative, which has been known to retain CA 19-9 antigenicity (2), to shorten fixation time; this allowed for good observation of CA 19-9 localization in the cells.

In order to fully investigate the CA 19-9 localization in ovarian cancer tissue by electron microscopy, a suitable comparison study in relation to ovarian cystadenoma is considered necessary. However, such reports are not yet forthcoming. In electron microscopic studies involving biliary epithelial cells in normal or chronic liver disease, it has been reported that the localization of CA 19-9 was confirmed in perinuclear spaces, endoplasmic reticula, Golgi apparatus, and plasma membranes (2). However, the localization of CA 19-9 in cases of primary ovarian cancer was observed in the Golgi apparatus and plasma membranes of cells which were rich in organelle, whereas reaction products were infrequently observed in the perinuclear spaces and en-
doplasmic reticula. Especially, in mucinous cystadenocarcinoma, reaction products were only recognized in plasma membranes. In endometrioid carcinoma, reaction products were observed in perinuclear spaces and endoplasmic reticula in cancer cells found to be degenerative. On the other hand, in metastatic ovarian cancer, the localization of CA 19-9 was recognized in perinuclear spaces and endoplasmic reticula in higher frequency compared with primary ovarian cancer and these cancer cells were not degenerative. The above results may indicate that the turnover of CA 19-9 in endoplasmic reticula and perinuclear spaces occurs too early for detection using the method applied in this study; only in cases where the transportation of CA 19-9 was impaired were the reaction products observed in perinuclear spaces and endoplasmic
In this study many pictures revealed positive vesicles which appeared to have released from the Golgi apparatus; this feature seems to be especially characteristic. In some pictures, the immunohistochemical method showed the presence of CA 19-9 only in the Golgi apparatus, without reaction products in the endoplasmic reticula. Thus, the Golgi apparatus is considered to be where immunoreactive CA 19-9 is first produced in cancer cells. It has been suggested that CA 19-9, which increased in the blood of cancer patients, has the same immunoreactivity with that detected in the blood of healthy human, but the structure of molecules of sugar chains of each were different.

In mucinous cystadenocarcinoma, the localization of CA 19-9 was not observed in the organelle, but reaction products large in both number and size were found in the glycocalyx. Since the cytoplasm was filled with a large number of mucus vesicles electron-microscopically, these cancer cells were regarded as cells which had already finished producing CA 19-9.

Reaction products were found not only in the intraductal materials, but also in collagen fibers directly under the basement membrane, which indicated that CA 19-9 expanded even to the peripheral stroma. In an immunohistochemical study of carcino-embryonic antigen (CEA), it was reported that when CEA appeared in the surrounding stroma, the CEA blood value increased (3). This indicates that an important factor for CA 19-9 increase in blood is due to the expansion of CA 19-9 to stroma. Since CA 19-9 was present in the organelle, the appearance of CA 19-9 in the cytoplasm of neoplastic cells indicates the secretory activity of these cells.

The above results indicated that CA 19-9 was useful as a tumor marker for ovarian cancer which originated in common epithelial cells.

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REFERENCES


